CHEMISTRY OF NATURAL PRODUCTS

DISSERTATION SUBMITTED FOR THE DEGREE OF

Master of Philosophy

IN

CHEMISTRY

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DEPARTMENT OF CHEMISTRY
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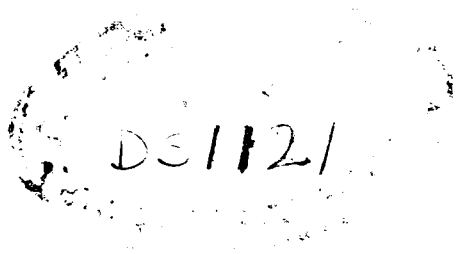
ALIGARH [INDIA]

MARCH 1987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This is to certify that the dissertation entitled, 'Chemistry of Natural Products' is the original work of the candidate and is suitable for partial fulfilment of the requirements for the degree of Master of Philosphy in Chemistry.

(M. Ilyas)
Supervisor

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Thanks are also due to all my lab. colleagues and friends.

Vikas

(Vikas Babu)

CONTENTS

Theoretical

About seventy to eighty per cent of the total Indian population, especially those living in villages and who can ill-afford to go in for costly modern medicine, rely on indigeneous medicinal systems to provide relief from diseases. Medicinal wealth of agricultural countries like India is enormous due to varied climatic conditions and the plants form the basis of our ancient Unani and Ayurvedic Systems of Medicine. Despite the spectacular advances, synthetic drugs have experienced set-back due to acute toxicity and side effects and this has created a revival of interest in the exploration and development of natural products for therapeutic purposes.

Search for therapeutic agents from natural sources has equipped modern medicine with a wide range of curative agents which despite the remarkable success in synthetic drugs have retained their importance. Plants have been the subject for the search of new or better drugs and new lead molecules for the drug development.

Researchers throughout the world are carrying out methodical surveys of untested plants in the hope of getting natural products like digoxin and reserpine to cure our many ills.

In common with the studies of natural products of all kinds, flavonoid chemistry has emerged from the undirected search of new compounds,and the establishment of their structures by conventional means.

The flavonoids are important to man not only because they contribute to plant colour but also because many members are physiologically active. The importance of flavonoidic compounds in the tanning of leather, the fermentation of tea, the manufacturing of cocoa and in the flavour qualities of foodstuff is well established[1a,b]. Certain flavonoids are among the earliest known natural dyestuffs[2a]. They are widely used in antioxidants for fats and oils[3a,b]. Among the physiological activities of flavonoids[1b,2b] include vitamin P activity, diuretic action, treatment of allergy, protection against X-rays and other radiation injuries, cure of frost bite, antibacterial activity, prophylactic action, oestrogenic activity, antitumour effects[4] and anticancer property[5].

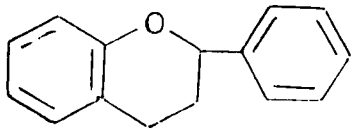
The flavonoids are generally polyphenolic compounds having C_6-C_3-C_6 carbon skeleton. Among flavonoids, the term assigned to this large class of natural substances, derivatives of 2-phenylchroman occupy an important position.

The term 'flavonoid' derives from the most common group of compounds, the flavones, whereas oxygen bridge between the ortho position of ring A and the benzylic carbon atom adjacent to ring B form a new γ-pyrone type ring. Such heterocycles, at different oxidation levels, are present in most plants. The flavane corresponds to the lowest oxidation level of ring C, and is taken as the parent structure for the rational nomenclature of this group of compounds.

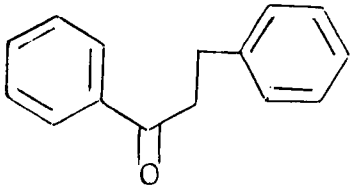
An oxygen bridge involving the central carbon atom of the C_3-chain occurs in a rather limited number of cases, where the resulting heterocycle is of the benzofuran type. The aurones belong to this structural group. The oxygen bridge is absent in chalcones and dihydrochalcones. Principal structural groups of natural flavonoids are shown in Fig.-1. The oxidation levels concern the three central carbon atoms: [+1O or -2H]= +1 oxidation unit.

Besides the carbon atom link, the flavonoids also have typical oxygenation pattern in their benzene rings. Ring A generally has three alternate oxygens at position 2', 4' and 6' in the open formula or, in other words ring A generally derives from phloroglucinol, compounds having more or less oxygens in their A ring are very seldom encountered. The ring A can be occasionally alkylated with methyl groups, prenyl or prenyl derived units or with glycosides. In contrast, ring B has, in most cases, a para-oxygen substituent, or two oxygens, para- and meta- with respect to propane chain. Compounds with non-oxygenated B ring or with one ortho-oxygen function are very rarely found. Compounds bearing three oxygens, one para- and two meta- are less frequent.

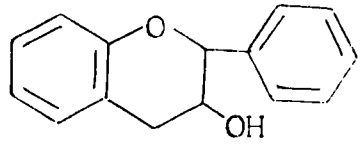
OXIDATION LEVEL

1. 

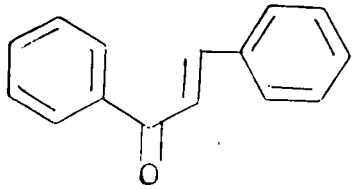
Flavan

2. 

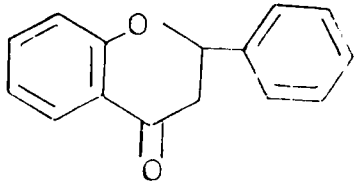
Dihydrochalcone



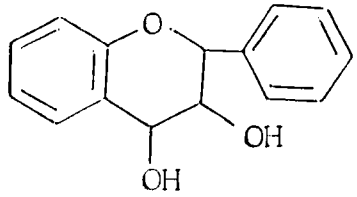
Flavan-3-ol (Catechin)

3. 

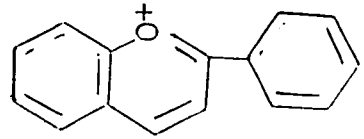
Chalcone



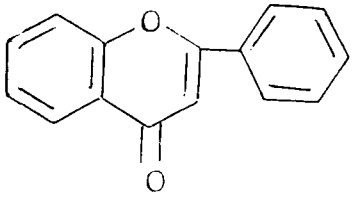
Flavanone



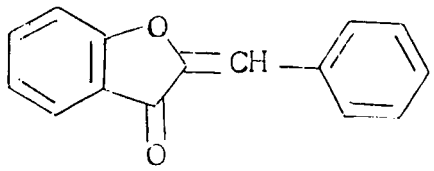
Flavan-3,4-diol



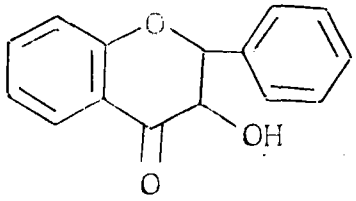
Flavylium salt

4. 

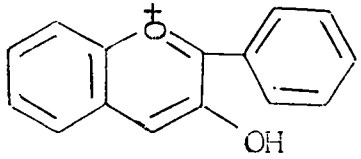
Flavone



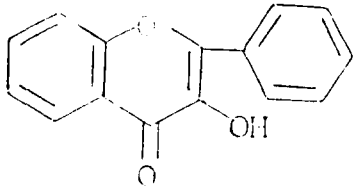
Aurone



Flavanonol



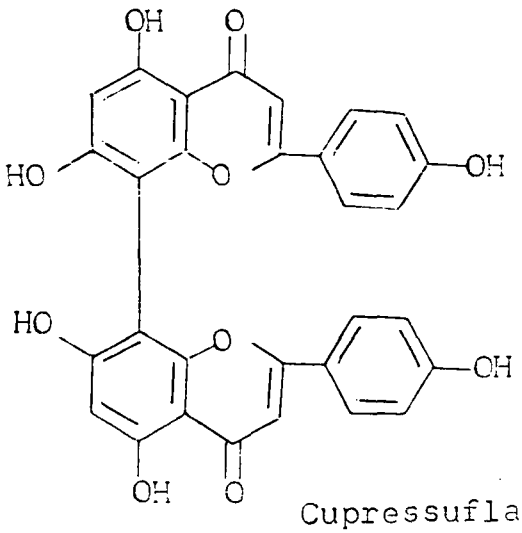
Anthocyanidin

5. 

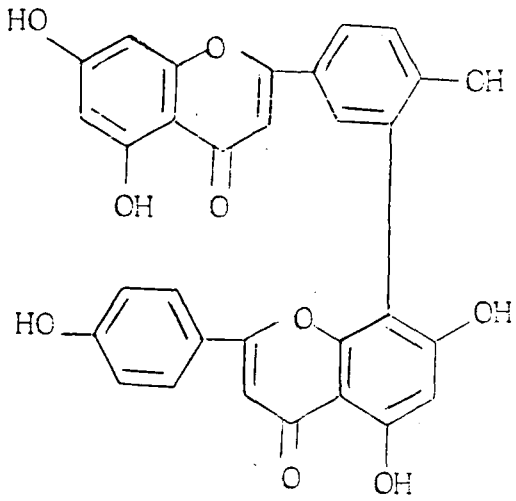
Flavonol

Fig.-1

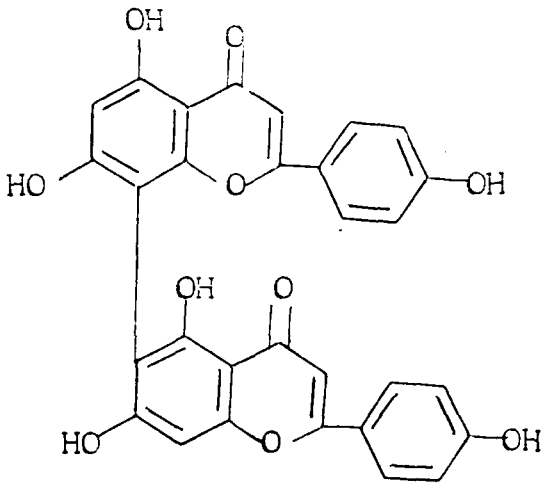
An interesting addition to the flavonoid class is 'biflavonoids'. Biflavonoids, which are generally derived from two apigenin or two naringenin or naringen-apigenin units, have mostly been isolated from gymnosperm. Depending upon the nature and the position of the linkage of the constituent monomeric units, all the C-C and C-O-C linked biflavonoids are classified into various families[6a], depending on the position of the linkage, two flavone units are shown in the Fig.-2a and 2b.



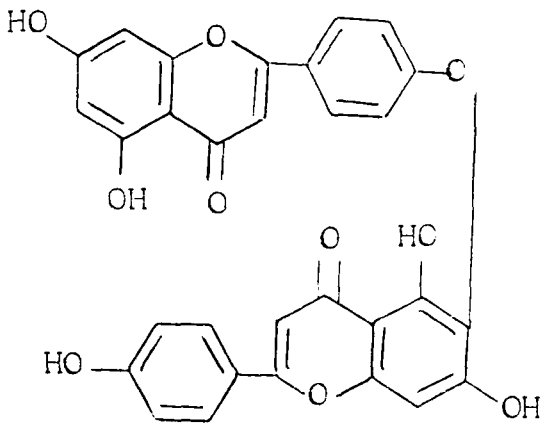
Cupressuflavone



Amentoflavone

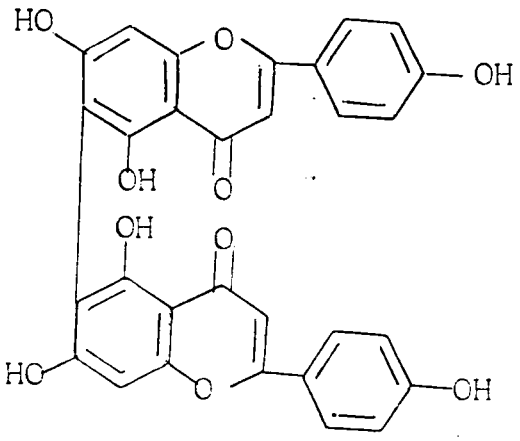


Agathisflavone

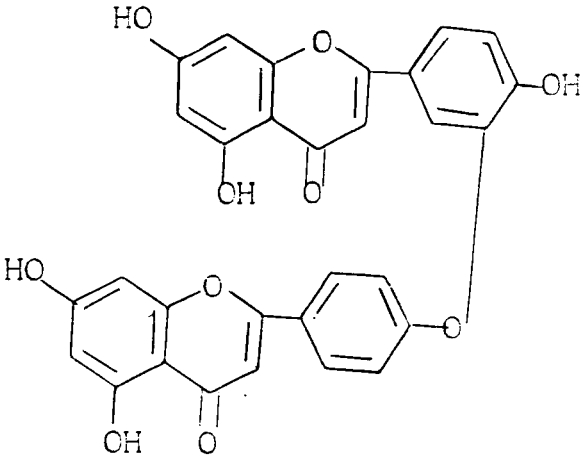


Hinokiflavone

Fig.-2a



Succedaneaflavone



Ochnaflavone

Fig.-2b

Biogenesis of Flavonoids

Chemical speculations on the mode of formation of the carbon-skeleton of this large class of natural products stimulated the interest in the biosynthesis of flavonoids. Although the origin of the carbon atoms of flavonoid is well known[7-9], the actual compounds that condense to yield the C_{15}-skeleton and the sequence of changes which result in the formation of a relatively diverse group of compounds, based on variation in the oxidation level of the C_3-portion of the molecules, are less well understood.

Basically ring A is formed by head-to-tail condensation of three acetate units while ring B as well as C_3-chain arise from a phenyl propanoid precursor derived from the shikimic acid pathway[10]. The involvement of acetic acid and substituted cinnamic acid has been confirmed through studies with labelled compounds, notably by Grisebach[11] and Geissman[12] (Fig.-3).

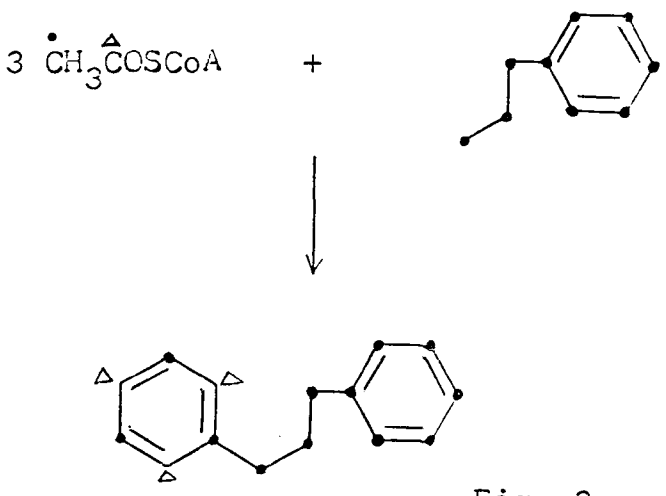


Fig.-3

However, a more detailed knowledge of this reaction and of the chemical nature of the immediate precursors obtained from the enzymic studies support the proposal that CoA ester of malonic acid and cinnamic acid are the substrates of an enzyme-mediated condensation (Fig.-4)[13,14], while no experimental evidence has been obtained, so far, regarding the possible intermediates in the formation of ring A from acetyl CoA, direct evidence for the reaction mechanism formulated in Fig.-4 has been obtained from the chemical degradation of the overall product formed from p-coumaroyl CoA and ^{14}C-labelled malonyl CoA with an enzyme-preparation from cell suspension culturesof parsley[15].

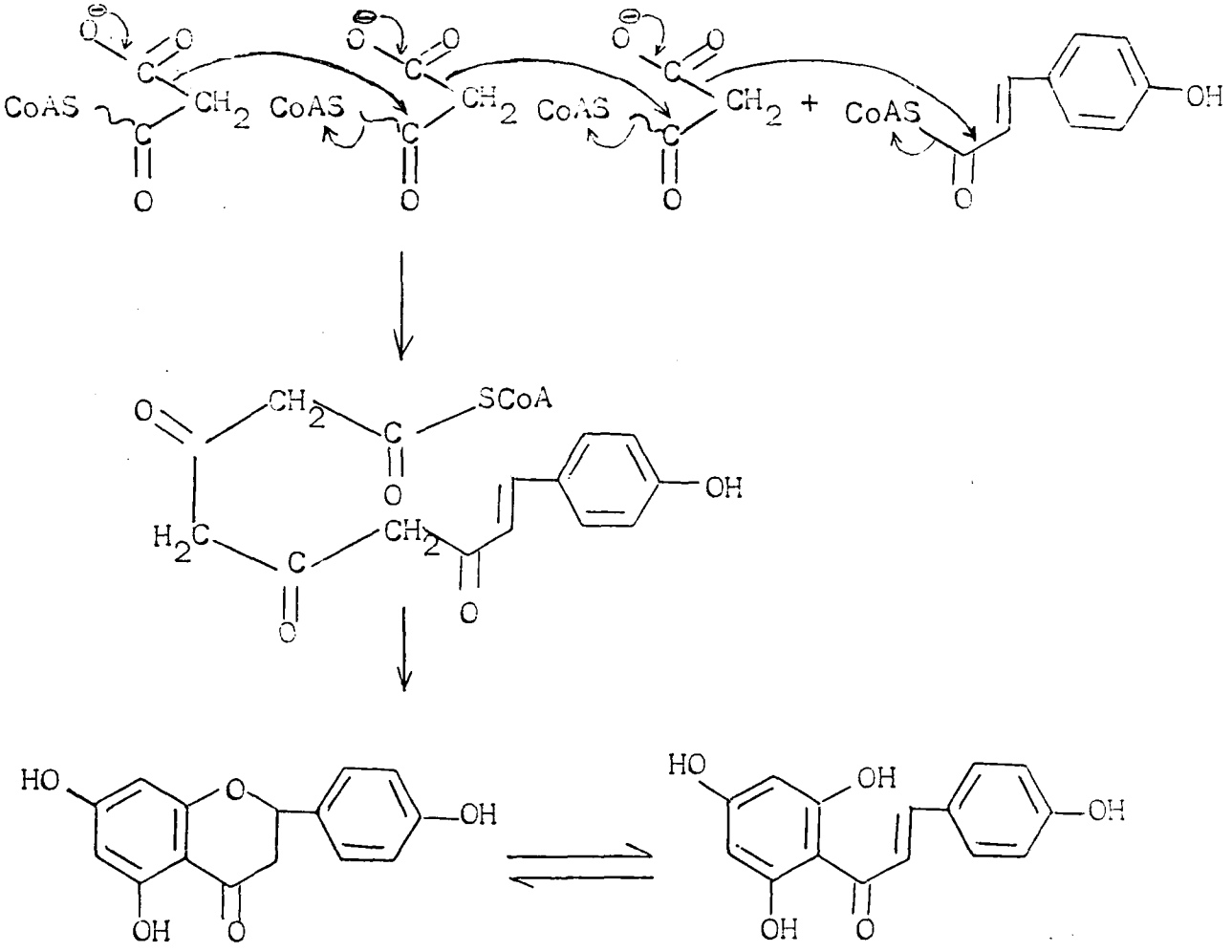


Fig.-4

There are still some doubts about the actual structure of the phenylpropane unit used by the plants as a starter for the process of polyketide condensation and then ring A. Most chemists now believe that the cinnamic acids (p-coumaric, and more rarely caffeic, ferulic and sinapic acids) are obligatory intermediates in the biosynthesis of most flavonoids[16].

It has been repeatedly demonstrated, using labelled chalcones[17,18] and flavanones[19,20] that these compounds are central intermediates from which most, if not all, other flavonoids originate. Conclusive evidence has not, so far, been obtained to answer the question whether chalcones or flavanones are the more direct precursors of the various flavonoids. There is good evidence for the *in vitro*[21] and *in vivo*[22] existence of an equilibrium between chalcones and the corresponding flavanones, the chalcone-flavanone interconversion is catalysed *in vivo* by an enzyme, chalcone flavanone isomerase, isolated from various plant sources.[16] The more important naturally occurring flavonoids are at the same or a higher oxidation level than flavanones, and many special hypotheses have been proposed to explain their genesis.

The first oxidative hypothesis for flavonoid biogenesis was proposed by Grisebach[23], who also made a detailed experimental study into the chemistry and biochemistry of flavonoids. The main feature of Grisebach's hypothesis was the formation of an epoxide chalcone (I), which could lead to flavonols, aurones, flavones and isoflavones, through plausible chemical mechanism (Fig.-5).

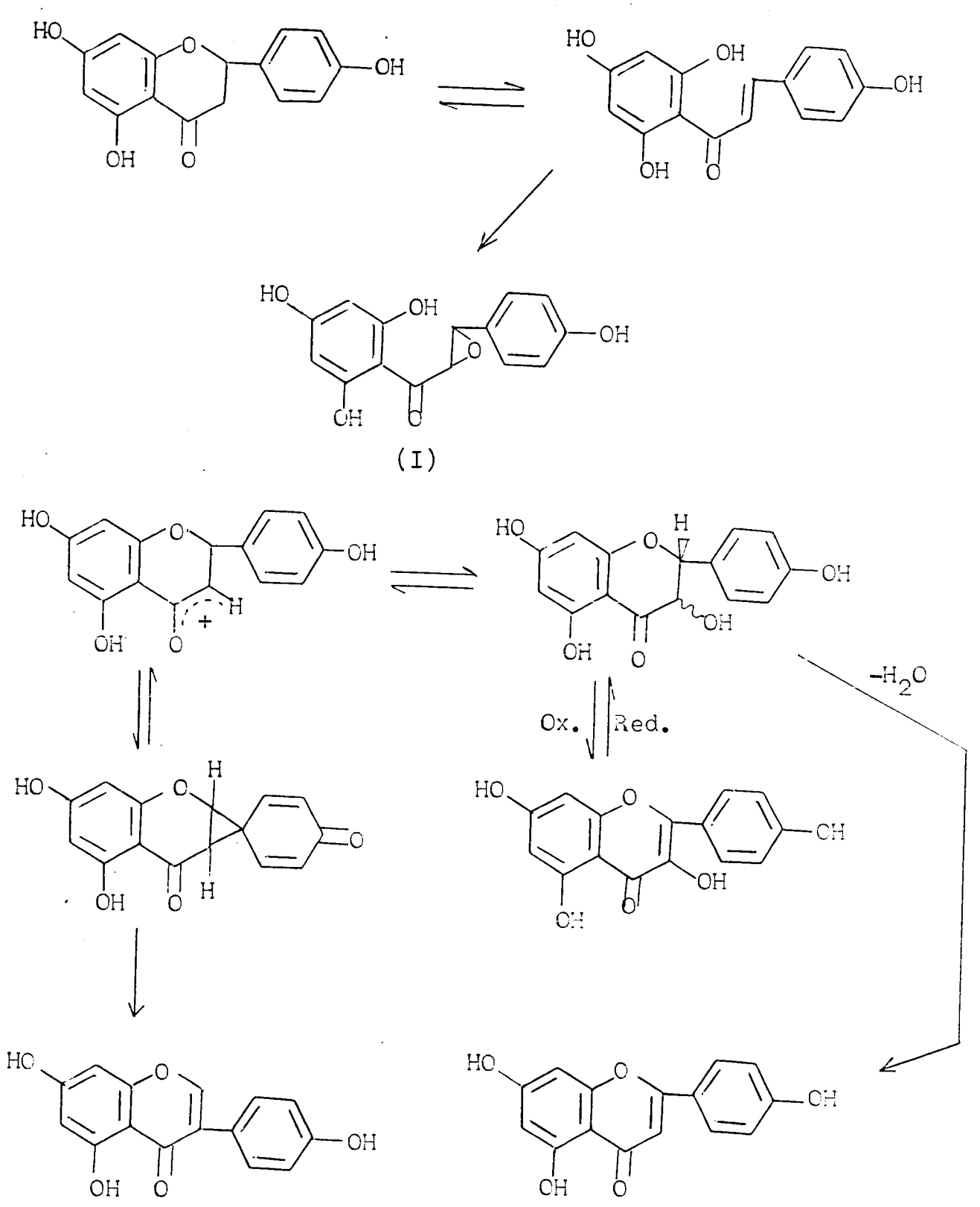

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Fig.-5

The weakest point of the epoxide hypothesis is that natural chalcone epoxides are as yet unknown. Although, synthetic 2'-OR chalcone epoxides are known[24], their epoxidation with H_2O_2 requires strongly alkaline conditions which are conditions totally different from those occurring *in vivo*[25,26].

There are alternative oxidation paths, which involve either the enolic form of the flavanone (II) followed by an attack by the equivalent of ^{+}OH and/or direct oxidation of a flavanone, to afford a cation at C-3 (III) which could be transformed to a flavone, flavanonol or isoflavone (Fig.-6)[27,28]. These hypotheses also give satisfactory explanation for the biogenetic correlations amongst the various flavonoids and, specially, for the very frequent presence of an oxygen atom in position C-3. However, the mechanisms proposed for the direct oxidation of flavanone to give a flavanone C-3 cation followed by reaction with ^{-}OH, and enolisation of the flavanone followed by attack by equivalent of ^{+}OH are doubtfully feasible *in vitro*.

Pelter[29] made a detailed experimental study into the chemistry of flavonoids and put forward a hypothesis, based on the phenolic oxidation of 4-hydroxychalcone (IV), which is supported by a large number of *in vitro* chemical analogies. Pelter suggested that a hydrogen (or hydride) abstraction from the 2- or 4-hydroxyl group in chalcone generates a radical (or cation) (V) which induces cyclisation as depicted in Fig.-7 to give isoflavone.

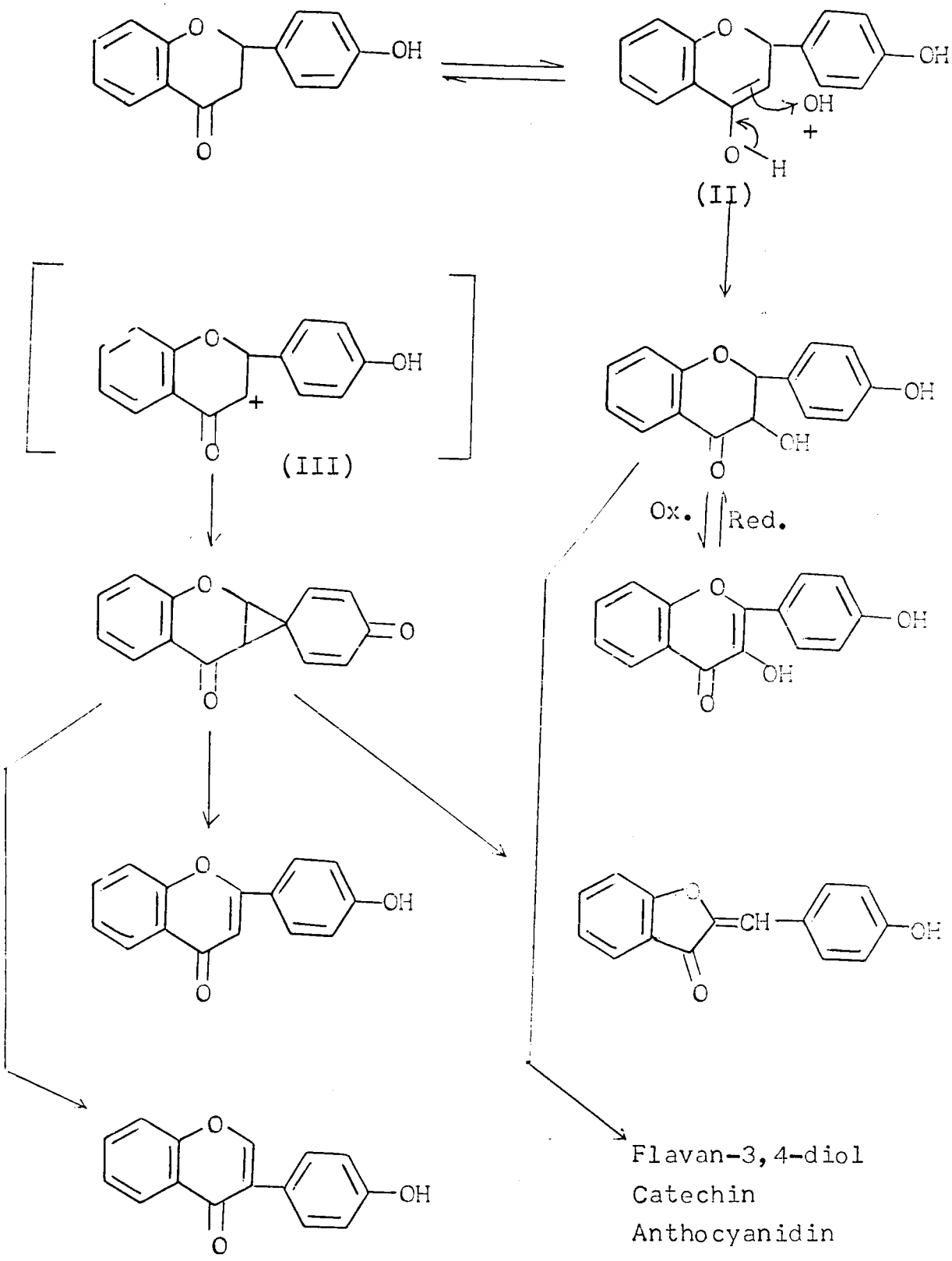


Fig.-6

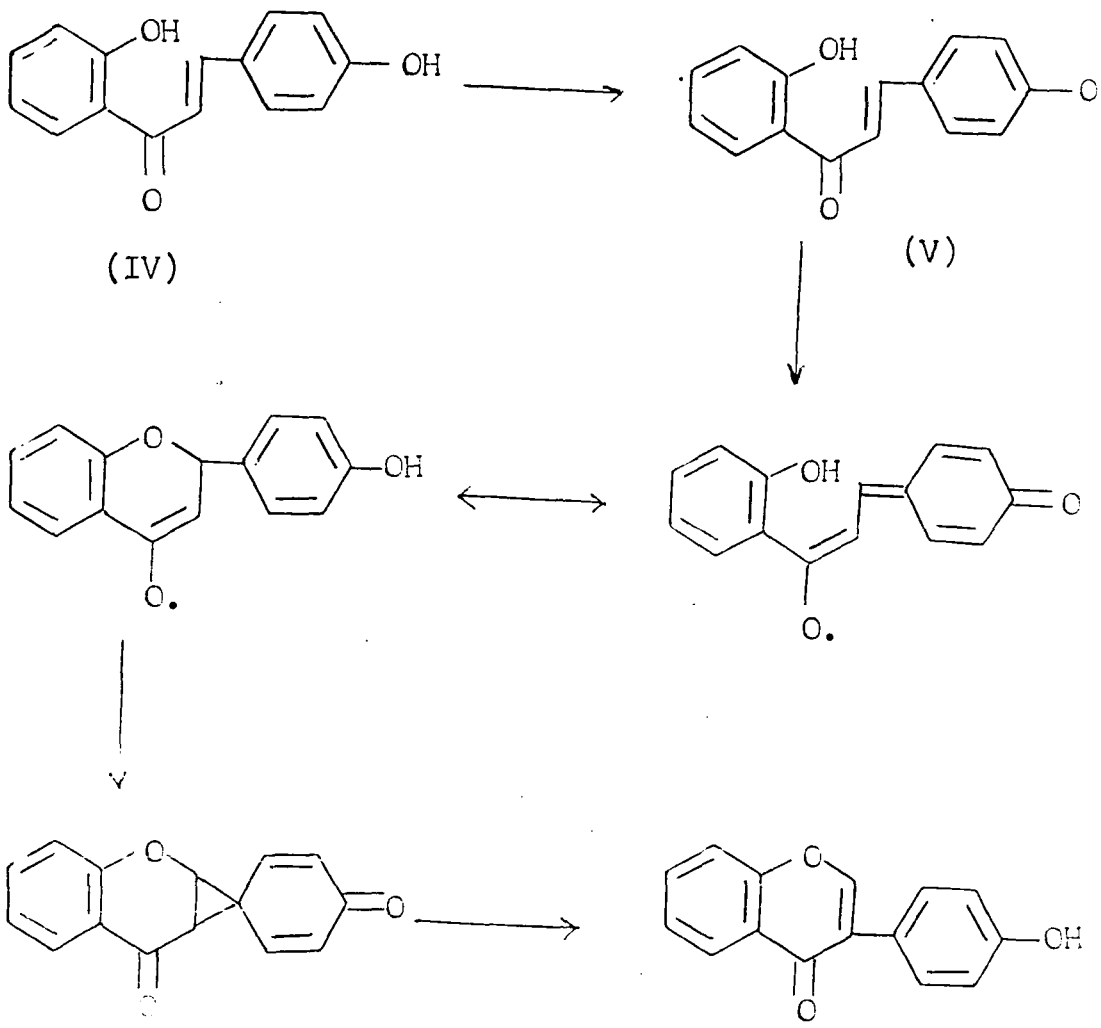


Fig.-7

Formation of flavones and aurones can be explained from the radical (V) as shown in Fig.-8. The formation of flavonols can be explained in terms of further oxidation of 4'-hydroxy group of either flavanonols or flavones (Fig.-9).

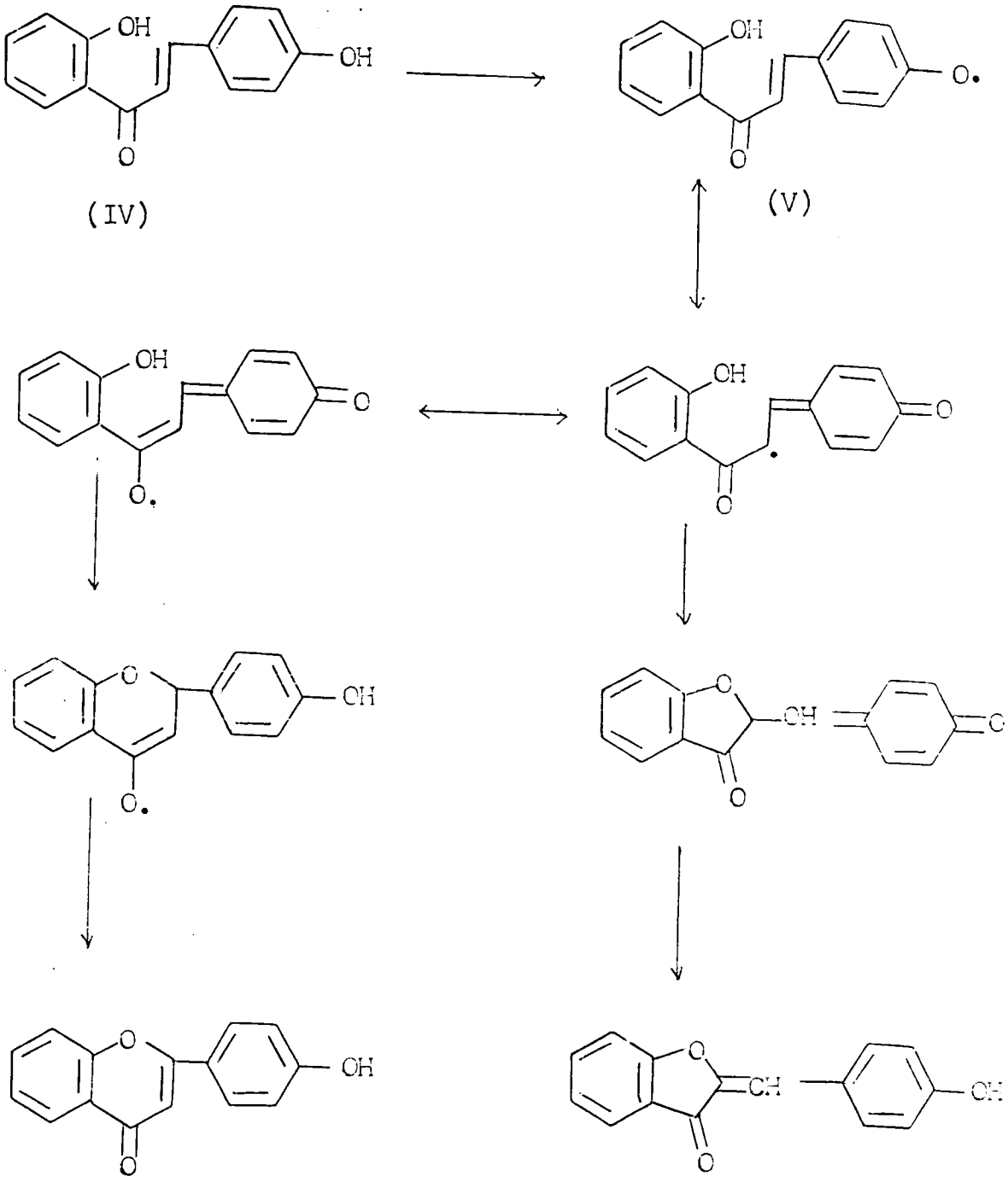


Fig.-8

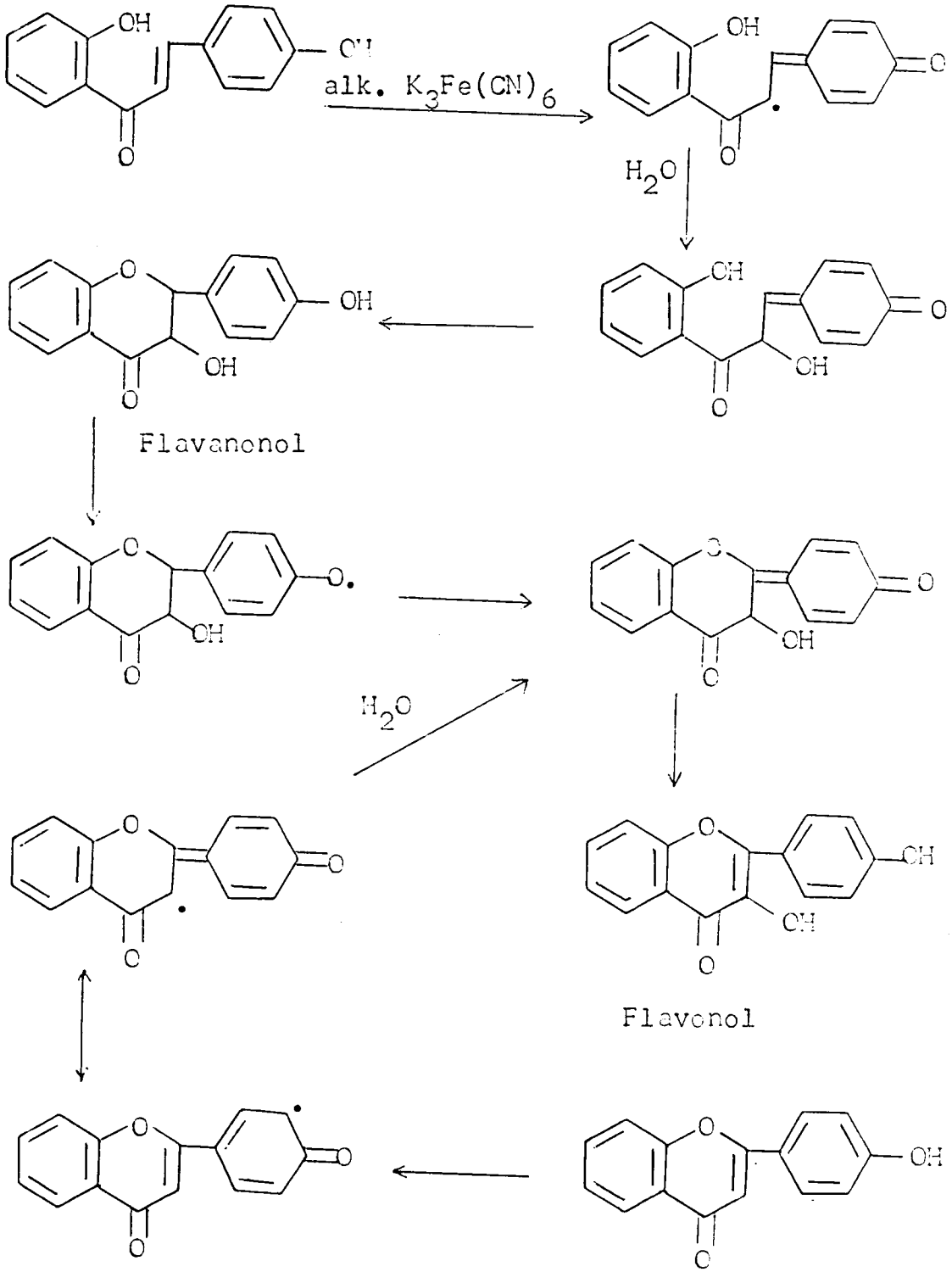
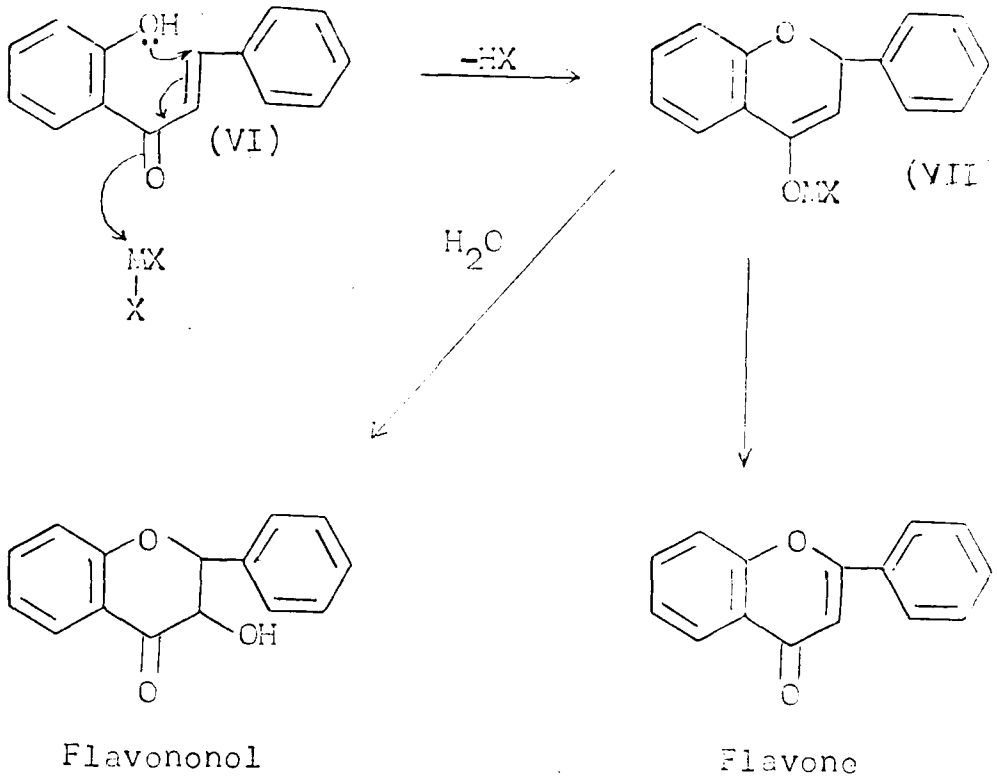


Fig.-9

The production of the flavonoids devoid of a hydroxyl group on the B-ring and above the flavanone oxidation level is not explained by Pelter's hypothesis. Pelter suggested that these compounds are probably produced directly from chalcones as shown in scheme-I. Cyclisation of the 2'-hydroxychalcone (VI) is initiated by a metal ion to yield the metal enolate (VII) followed by an oxidative loss of the metal yield flavone. If attack on the C-3 of metal enolate (VII) were by water, then flavanonol would result.

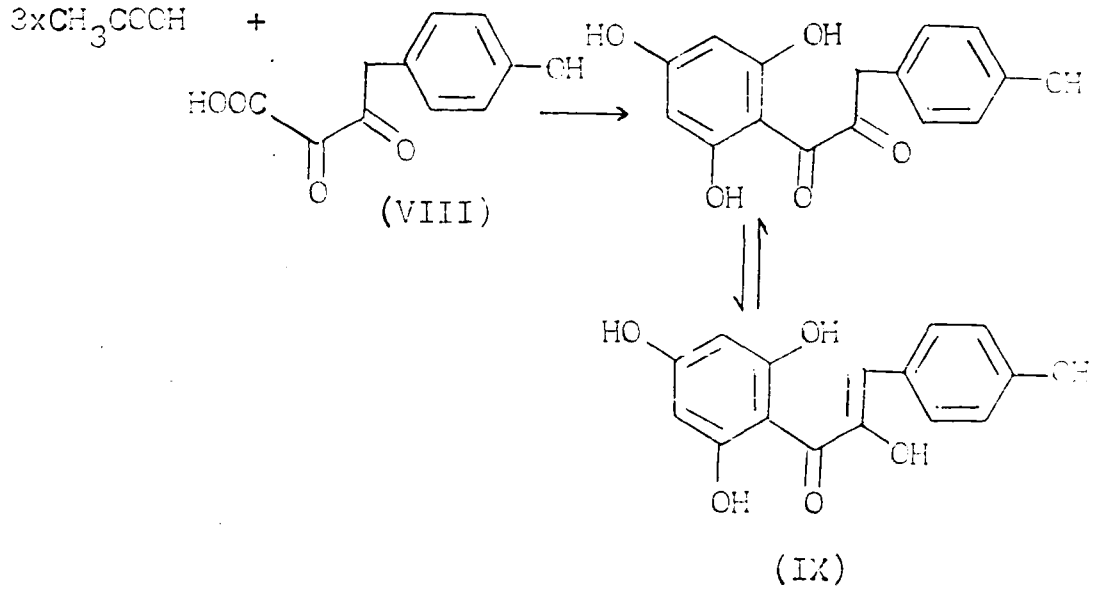


Flavononol

Flavone

Scheme-I

Roux and Ferreira[30] have proposed another hypothesis for the flavonoids biosynthesis from α-hydroxychalcone (IX) which probably originates from p-hydroxyphenylpyruvic acid (VIII)[31,32] and malonate (or acetate) units. The Roux theory is supported by the large natural distribution of α-hydroxychalcones[33,34].



(IX)

The formation of both 2,3-cis- and 2,3-trans-flavanonols can be explained by the cyclisation of the enolic from of α-hydroxychalcones. Subsequent reduction of these flavanonols leads feasibly to 2,3-cis- and 2,3-trans-flavan-3,4-diols and eventually to corresponding flavan-3-ols (Fig.-10). These classes of compounds form flavonols and anthocyanidins by oxidation and elimination reactions, respectively.

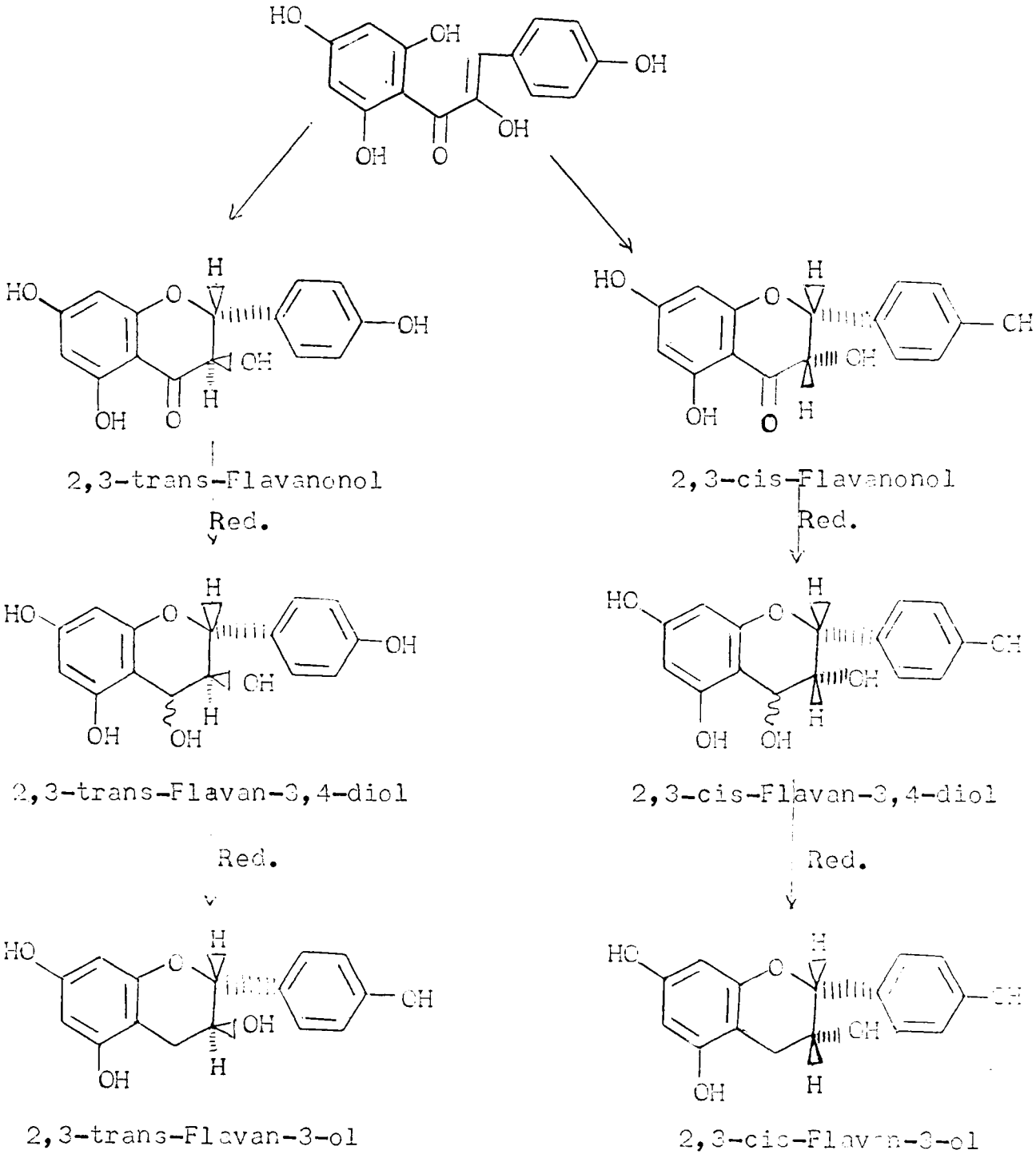
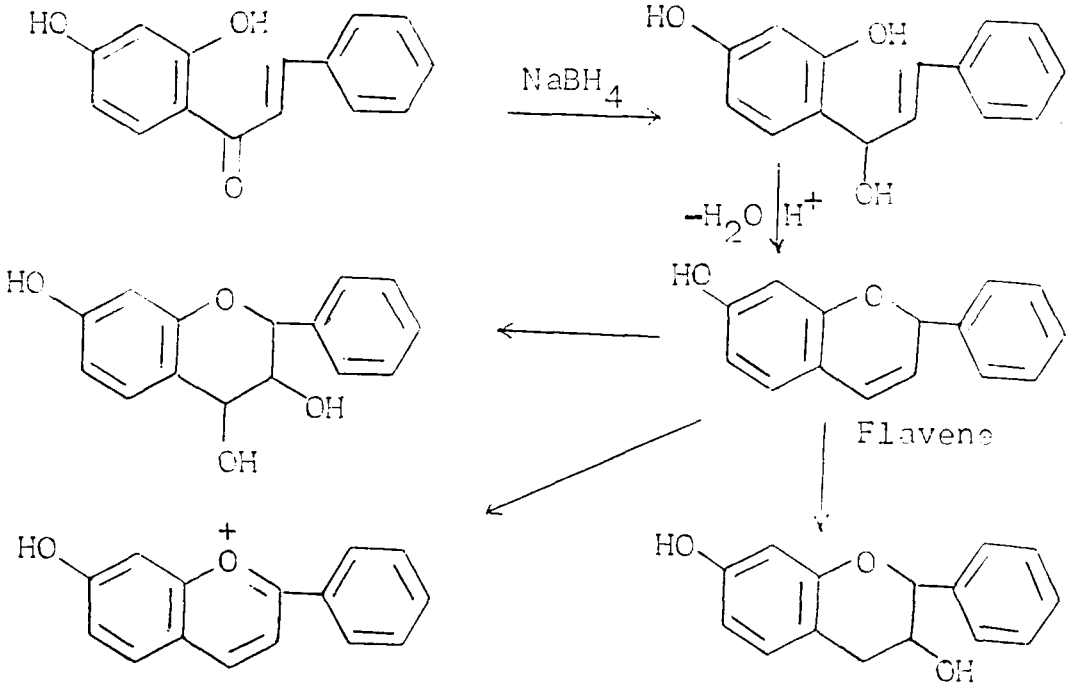

2,3-trans-Flavanonol
2,3-cis-Flavanonol
Red.
Red.
2,3-trans-Flavan-3,4-diol
2,3-cis-Flavan-3,4-diol
Red.
Red.
2,3-trans-Flavan-3-ol
2,3-cis-Flavan-3-ol

Fig.-10

A recent hypothesis (Fig.-11)[35] postulates a simultaneous phenol oxidation of the two aromatic rings of the chalcone intermediate, followed by an intramolecular coupling, according to a scheme very common in plants. Intermediates such as (X) and (XI) (oxonium salts) should be formed, which could lead to aurone, flavone, flavanonol and isoflavone.

Reduction of flavanonols is assumed in the biogenesis of flavonoid structures in low oxidation level, but other possibilities also exist. Thus, as shown by Clark-Lewis and co-workers[36,37], sodium borohydride reduction of chalcones gives flavenes which can conceivably also serve as precursors of leucoanthocyanidins, anthocyanidins and catechins[38].



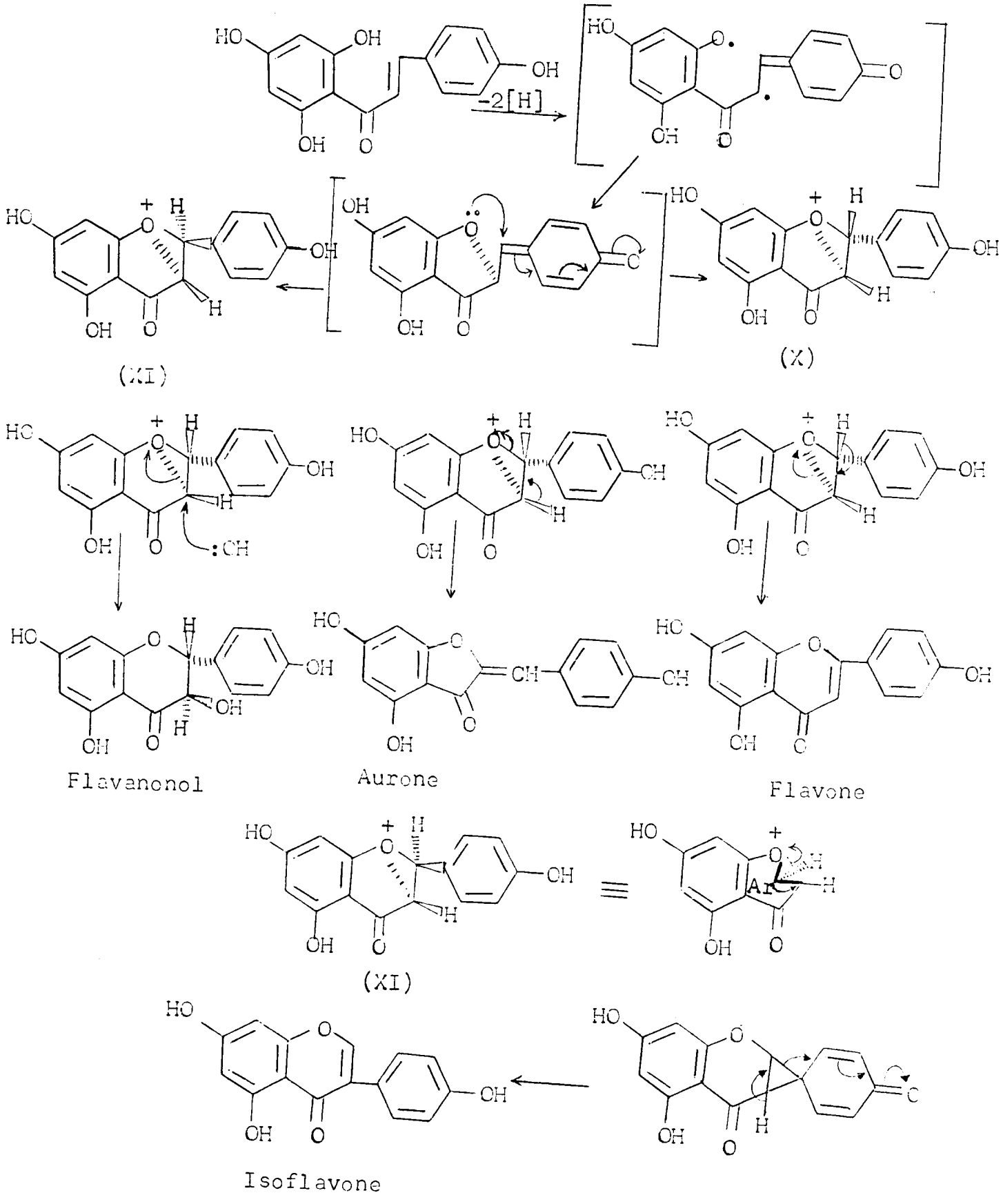
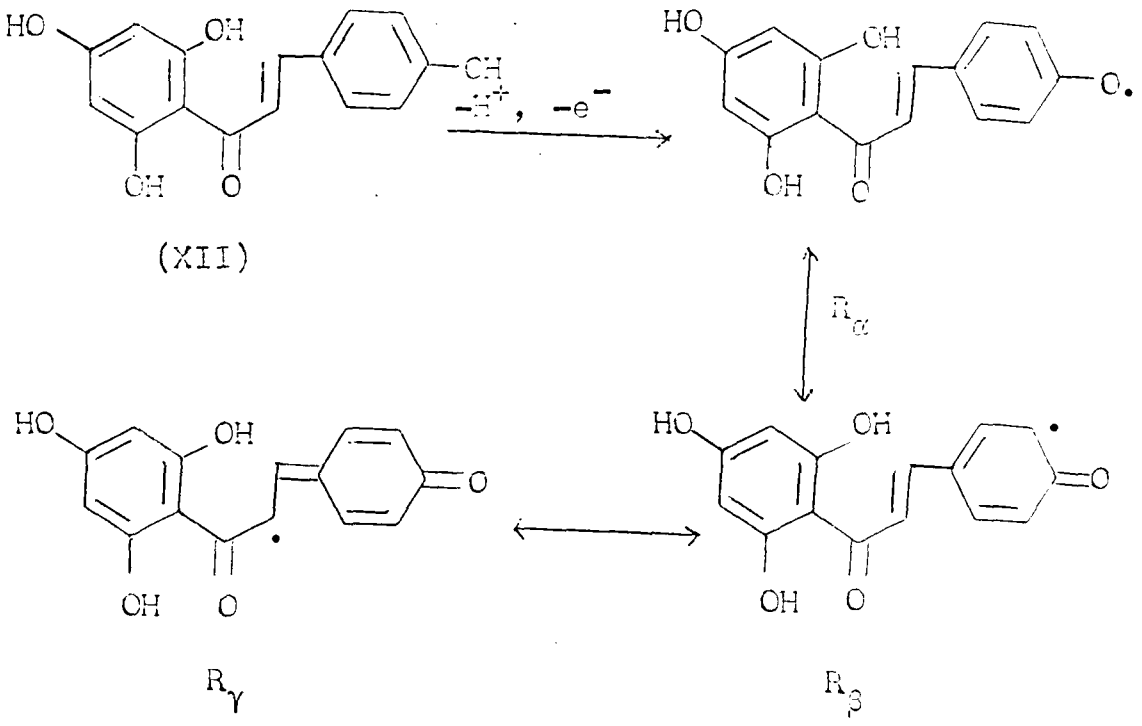


Fig.-11

The mechanism of formation of ring C in anthocyanidins has still many obscure points. Haslam's hypothesis[39] on the anthocyanidin and catechin biosynthesis is the most widely accepted one and strictly correlates the biosynthesis of anthocyanidins with catechins (Fig.-12).

The formation of biflavonoids, despite the range of biflavanoids now known, may be explained in terms of oxidative coupling of two chalcone units and subsequent modification of C_3-chain[6a]. Abstraction of an electron from the C-4 anion of naringeninochalcone (XII) affords radical which may be represented by the canonical formulae R_α, R_β and R_γ.



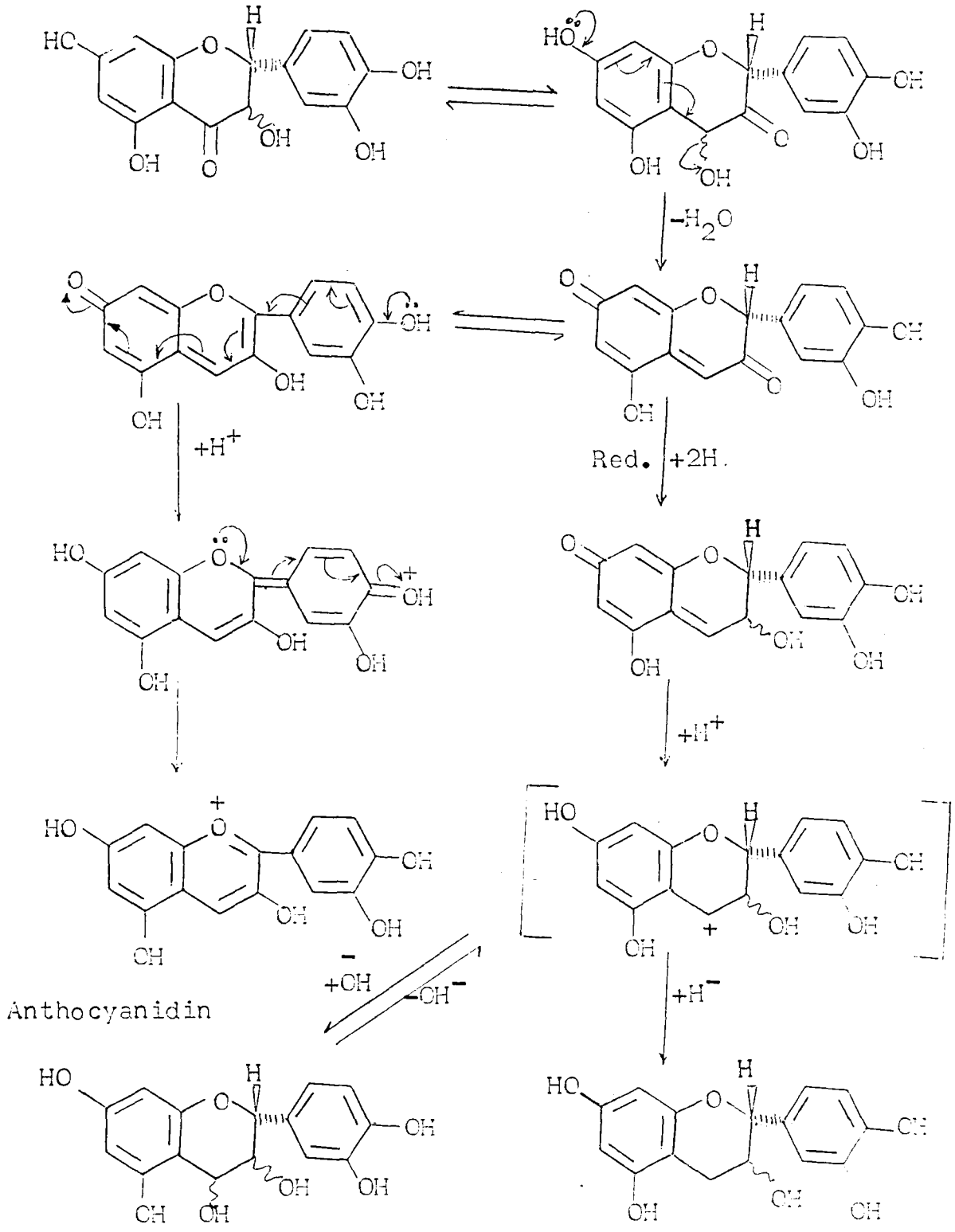
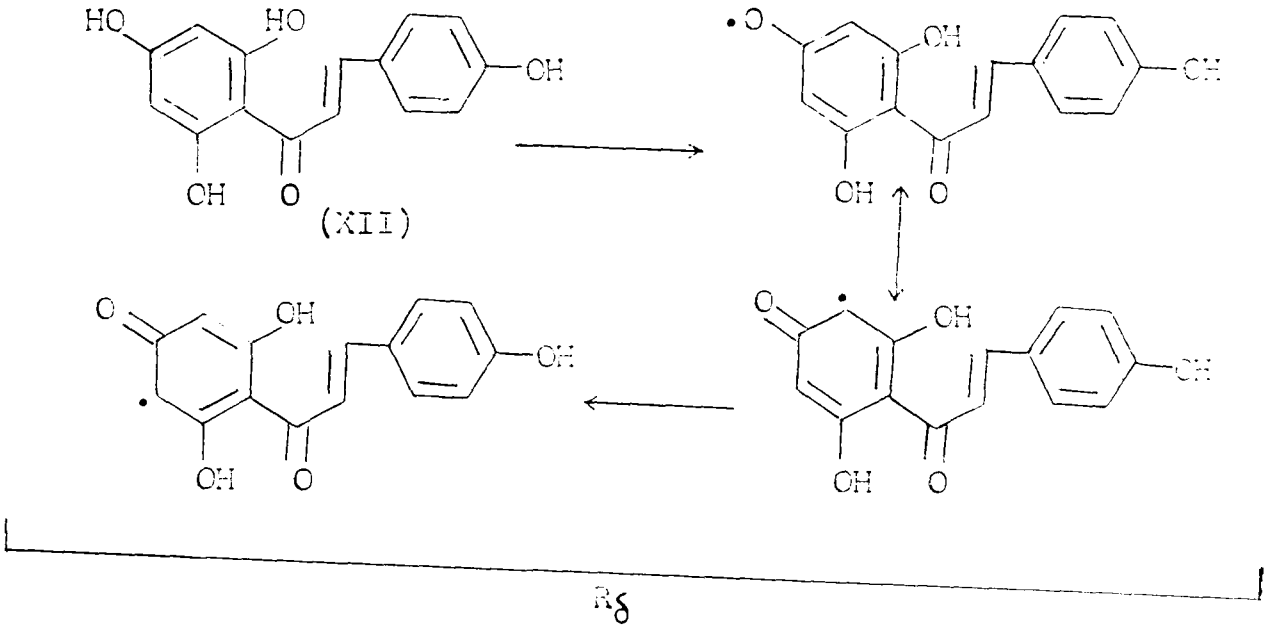


Fig.-12

While the abstraction of an electron from the C-4' anion of (XII) will give another radical which may be represented by several canonical formulae. However, the only canonical formulae which are important in the biosynthesis of most of biflavonoids are R_δ. The formation of precursors of all naturally occurring biflavonoids can be explained by the appropriate pairing of these radicals.



Structural Elucidation of Flavonoids

The structural elucidation of flavonoids has been discussed in detail in some recent reviews[40] and monographs[6,41]. However, some of the techniques frequently used by us and included in the discussion of this dissertation need mention here.

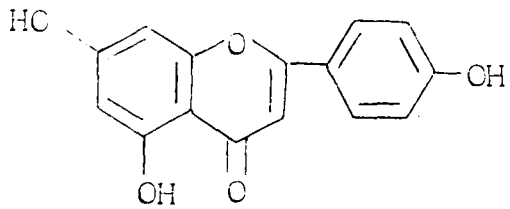
Nuclear Magnetic Resonance Spectroscopy

The application of the n.m.r. spectroscopy proved to be the most powerful tool in the structure determination of flavonoids. The valuable contribution in this field has been made by Batterham[42], Mabry[43], Massicot[44], Clark-Lewis[45], Kawano[46] and Pelter and Rahman[47]. Early work on the ^{1}H-n.m.r. spectroscopy of flavonoids was hindered by lack of their solubility in $CDCl_3$ and CCl_4. Progress was made following the introduction of Me_2SO-d_6[48], but the most significant advance arose from the conversion of flavonoids into their more soluble trimethylsulyl ethers[49].

The chemical shifts of the protons of rings A and B prove to be independent of each other, but are affected by the nature of ring C[48]. The peaks arising from ring A in most flavonoids occur upfield from other peaks and can readily be recognised.

The most commonly occurring hydroxylation pattern in natural flavonoids is 5,7,4'-trisubstituted system (XIII) in which, owing to the symmetrical substitution, ring B protons

appear as superimposed doublets (J=9 Hz) corresponding to an A_2B_2 system and ring A protons as AB doublets (J=2.5 Hz). In other cases, however, the interpretation is not so simple owing to the superimposition of signals and appearance of complex multiplicities of protons of an ABX or ABC system.



(XIII)

Considerable variations are generally found for the chemical shifts of the ring C protons among various flavonoid classes[41]. For example, the C-3 proton in flavones gives a sharp singlet near ð 6.3, the C-2 proton in isoflavones is normally observed at about ð 7.7, while the C-2 proton in flavanones is split by C-3 protons into a doublet of doublet (J_{cis}=5 Hz, J_{trans}=11 Hz) and occurs at about ð 5.2. The signals for the two C-3 protons in flavanones appear as a pair of quartets ($J_{H-3a-3b}$=17 Hz) near ð 2.7-3.0. However, they often appear as two doublets since two signals of each quartet are of low intensity, the C-2 proton in flavanonols appears near ð 4.9 as a doublet (J=11 Hz) coupled with C-3 proton which comes at about ð 4.2 as doublet.

Solvent induced shift has been used for assigning the position of methoxyls in methoxyflavones. By measuring the n.m.r. spectra, first in $CDCl_3$ and then in C_6D_6, Wilson *et al.*[50] found that the size of the benzene induced shift (Δ) of certain methoxyl signals was to some extent indicative of the position of the methoxy group in the flavone nucleus.

A more recent innovation in this field is that of lanthanide induced shift[46]. The technique is extremely helpful in establishing the internuclear linkage of biflavonoids and also for the distinction of A and B ring methoxyl signals.

Mass Spectrometry

Electron impact mass spectrometry (EI-MS) serves as a valuable tool in the structure determination of flavonoids, especially when only very small quantities of the compounds are available. It has been applied successfully to all kinds of flavonoids.

Most flavonoid aglycones yield intense peaks for the molecular ion ($M^{+\cdot}$). In addition, peaks for $(M-H)^+$ and, when methoxylated, $(M-CH_3)^+$ are usually the major peaks. The most useful fragmentations in terms of flavonoid identification are those which involve cleavage of intact A-and B-ring fragments[6b].

Two common fragmentation patterns of flavonoids are, an exception being chalcones which undergo direct fission on each side of carbonyl group, retro-Diels-Alder (RDA) cleavage, pathway I and pathway II (Chart-I). Pathway I (RDA) process affords two different ions designated as $A_1^{+\circ}$ and $B_1^{+\circ}$, the ratio of the two being indicative of the charge distribution within the parent ion. In contrast, pathway II yields predominantly a single charged species, B_2^+. These two fragmentation processes are competitive and the combined intensities of the B_2^+ and $[B_2\text{-CO}]^+$ ions are approximately inversely proportional to those of $A_1^{+\cdot}$ and $B_1^{+\cdot}$ and the series of ions derived from them (Chart-I).

Flavones were among the first flavonoids to be analysed by mass spectrometry[51,52]. Although molecular ion, $M^{+\cdot}$, is the base peak for most of the flavones, the fragment $(M\text{-CO})^{+\cdot}$ and pathway I fragments $A_1^{+\cdot}$ and $B_1^{+\circ}$ are usually prominent. An ion $(M\text{-}1)^+$ is often found in the mass spectra of flavones, its origin, however, is obscure. Pathway II fragment, B_2^+, though usually found, is not much intense.

In the case of flavanones, typical fragmentation by RDA process yields ions which correspond to the same $A_1^{+\circ}$ and $(A+1)^+$ ions, observed for flavones, (Chart-I); however, the B-ring ion contains an ethylene group[53,54].

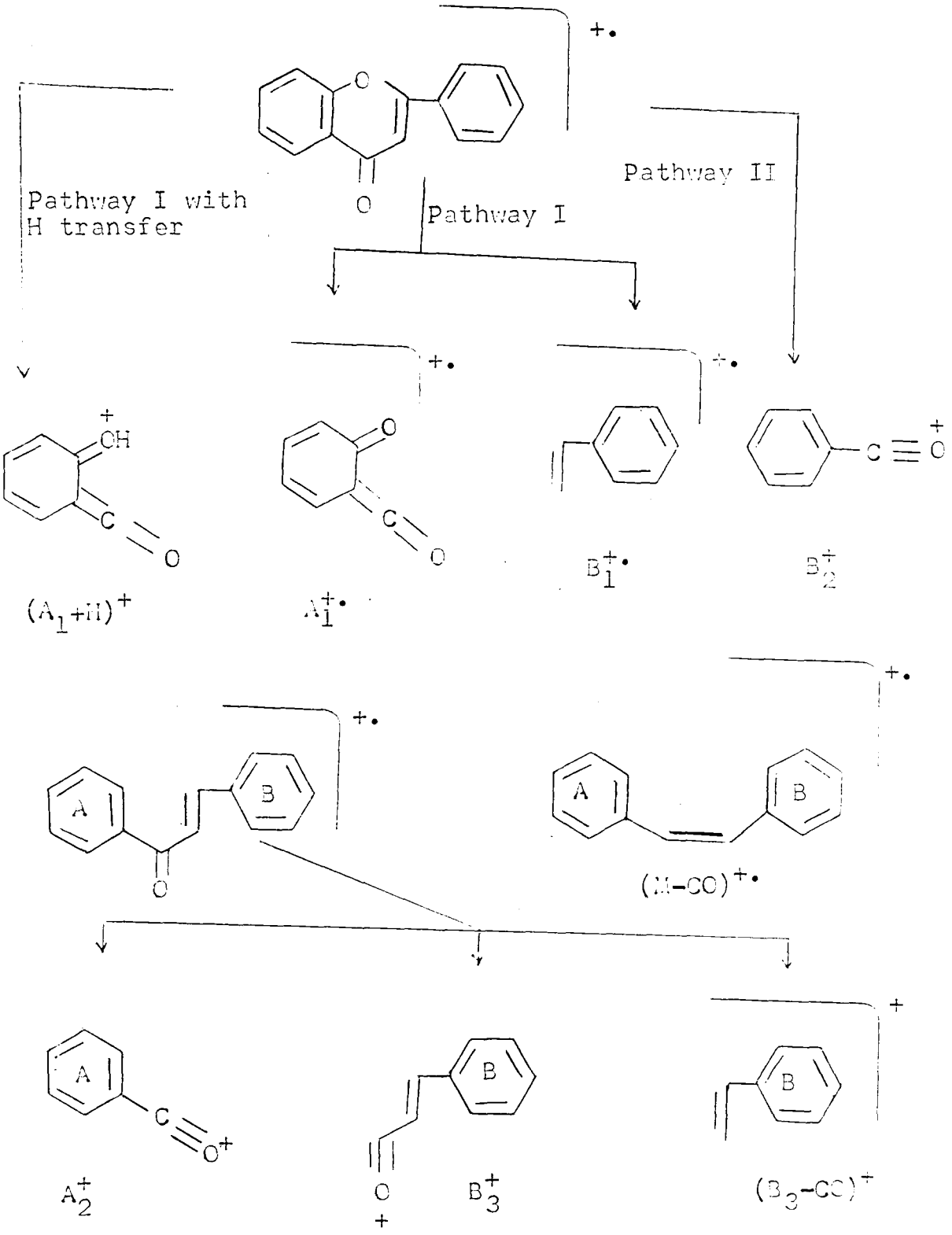
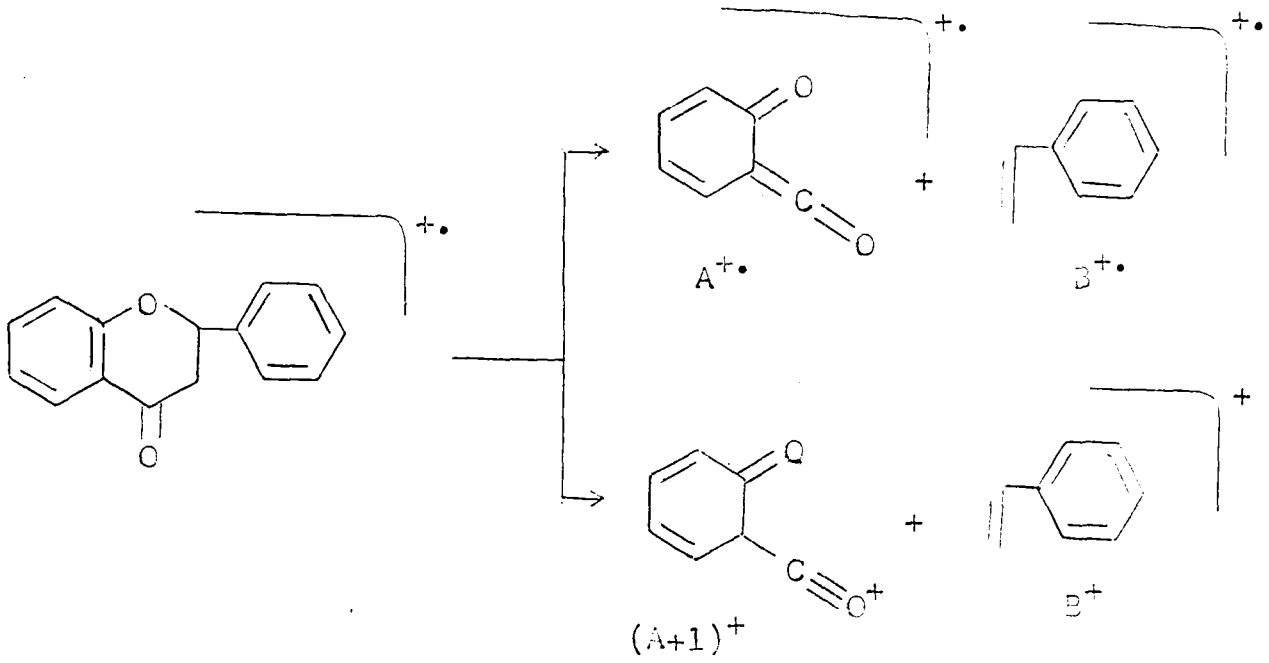
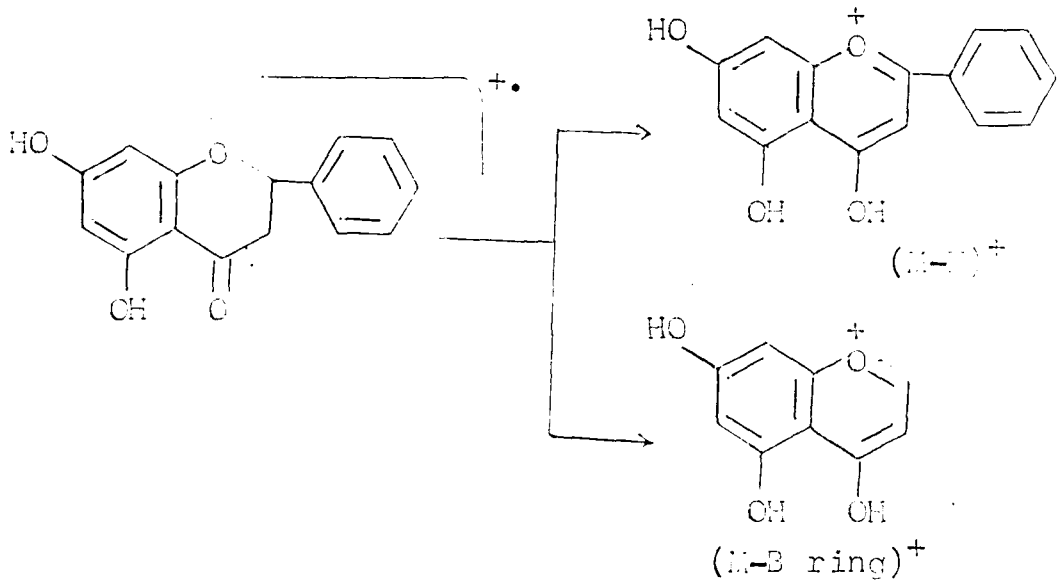

Pathway I with
H transfer
Pathway I
Pathway II
$(A_1+H)^+$
$A_1^{+\bullet}$
$B_1^{+\bullet}$
B_2^+
$(M-CO)^{+\bullet}$
A_2^+
B_3^+
$(B_3-CO)^+$

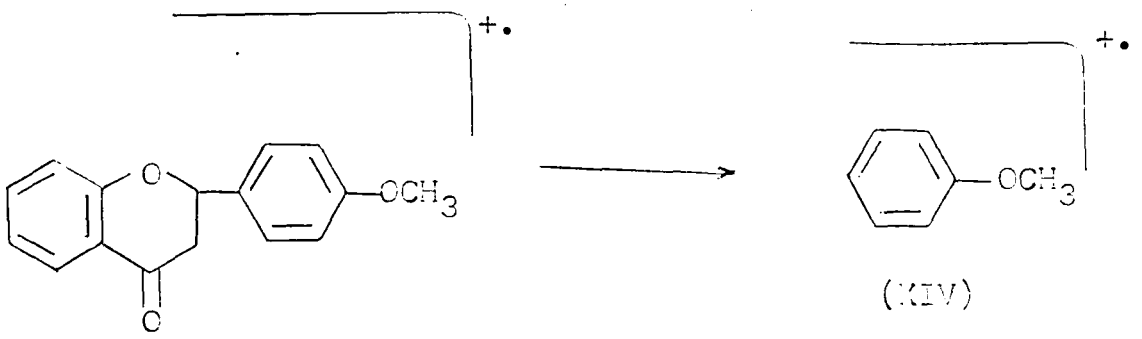
Chart-I



Another diagnostic fragmentation, that helps in structure determination of flavanones, is the loss of either a hydrogen or an aryl radical to produce $(M-1)^+$ and $[M-(B\text{-ring})]^+$ ions.

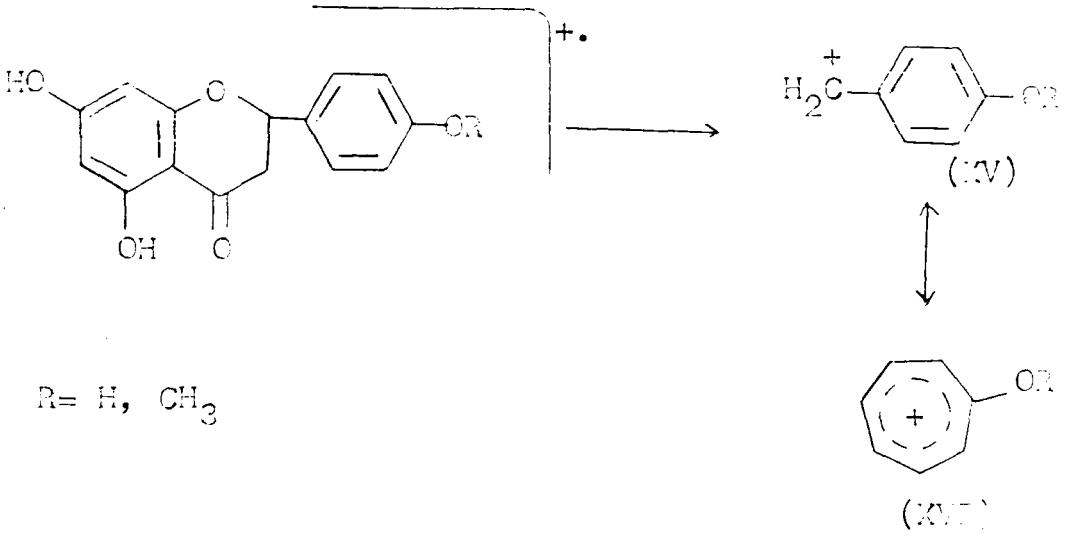


A moderately intense B-ring ion (XIV) is found in the case of 4'-methoxyflavones, which is formed by the fission of the B-ring from the molecular ion accompanied by a hydrogen transfer.



(XIV)

The presence of a hydroxyl or a methoxyl group at C-4' facilitates, by the enhanced resonance stabilisation of the molecular ion, the formation of p-hydroxybenzyl or p-methoxybenzyl cation (XV), respectively. The prominence of this ion may be associated with its rearrangement to a tropolium structure (XVI).



(XV)

R= H, CH_3

OR

(XVI)

Chalcones give strong ions for $M^+_{\bullet}$, $(M-H)^+$ and $(M-CH_3)^+$ (for methoxychalcones). However, most diagnostic fragments result by the fission on either side of the carbonyl group. The relative intensities of these ions, designated as A_2^+ and B_3^+ (Chart-I) and of those derived from them, depend upon the substitution pattern of the chalcone[54,55].

It has been established, in the case of 2'-hydroxy-chalcones, that an equilibrium exists between chalcone and flavanone and the ions derived by fragmentation of both the chalcone and its corresponding flavanone are found. In some cases, however, the cleavage of the chalcone adjacent to the carbonyl group is much faster than the isomerisation to flavanone and thus the spectrum of the chalcone predominates. It has been emphasised that, in most cases, it is difficult to determine with certainty from the mass spectral data whether chalcone or flavanone was originally present.

Mass spectrometry has also been very useful in the structure elucidation of biflavonoids. Biflavonoids have mostly been studied as their permethylated derivatives[40]. In general, two flavonoid units of a C-C linked biflavonoid fragment by the pathways which are well defined for the corresponding monoflavonoids. Some A- and B-ring fragments are exactly the same as those observed for the monoflavonoids, while others are typical A- and B-ring fragments except that they have an intact flavonoid unit attached to them. The C-O-C linked biflavonoids undergo fission on both the sides of the ether linkage to yield ions which undergo further fragmentation. Doubly charged ions are usually present. The mode of fragmentation of amentoflavone hexamethyl ether, a C-C linked biflavone, is shown in Chart II.

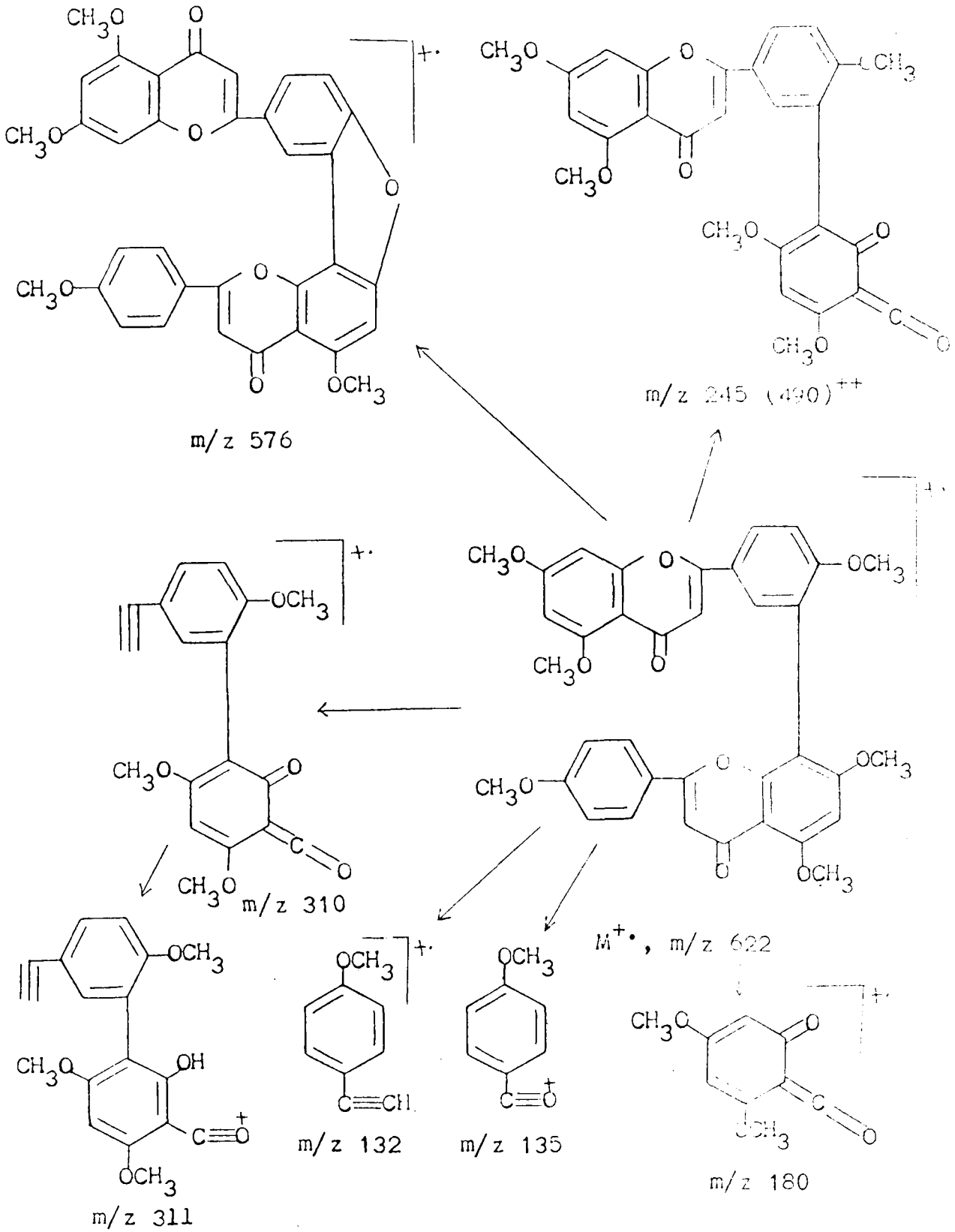


Chart-II

^{13}C-n.m.r. Spectroscopy

The ^{13}C-n.m.r. spectroscopy has now developed as one of the most useful techniques in the characterisation of natural products. It has been successfully applied in the field of flavonoid and the structures of mono- and biflavonoids have been elucidated with the help of ^{13}C-n.m.r. spectroscopy[56].

Carbon-13 with a natural abundance of only 1.1% and a smaller magnetic moment than ^{1}H was for many years a nucleus relatively inaccessible to n.m.r. spectroscopists. However, with the advent in recent years of Pulred n.m.r. and Fourier Transform (FT) analysis, ^{13}C-n.m.r. spectroscopy has become more readily available. It should be stressed, however, that ^{13}C-n.m.r. is in no sense superseding ^{1}H-n.m.r., but is complementary to it, informations gained related to the carbon 'back bone' of the molecule while ^{1}H-n.m.r. gives information about the structural environment of each proton.

Carbon-13 resonances in flavonoids occur over a range of 0-200 ppm downfield from TMS compared with the range of only 0-10 ppm for ^{1}H resonances. For this reason ^{13}C-n.m.r. spectra are much more highly resolved than are ^{1}H-n.m.r. spectra. In proton decoupled spectra each carbon atom is represented by one line and its chemical shift is determined preliminarily by the electron density at that carbon atom. In contrast to the value of

integration in ^{1}H-n.m.r. spectroscopy, integration of ^{13}C-n.m.r. spectra is largely uniformative unless spectral precautions are taken.

The signals for the carbonyl carbon and oxygenated aromatic carbons resonate at lowest field, whereas those at highest field will represent non-oxygenated aliphatic carbons. ^{13}C-resonances for the o/p-oxygenated aromatic carbons appear in the range 155-165 ppm while those for meta-oxygenated carbons appear in the 130-150 ppm. Signals for the non-oxygenated aromatic carbons appear in the region 125-135 (no o/p-oxygenation), and 90-125 (with o/p-oxygenation). Signals for aromatic methoxyl and acetyl carbons resonate in the range 55-60 and 20 ppm, respectively.

There are marked changes in the ^{13}C-n.m.r. of flavonoids on acetylation of free phenolic hydroxyl groups and can be used to locate the hydroxyl function on the aromatic rings. The signal of the hydroxylated carbon moves upfield by 6.6-15.6 ppm on acetylation, the ortho- and para-carbon signals are moved downfield by 7.1-12.7 and 2.0-7.9 ppm, respectively, the meta-carbon signals are slightly affected (+0.9 to -4.3 ppm). The effect of methylation of free phenolic hydroxyls is generally smaller, opposite and rather variable. The signal of the hydroxylated carbon shifts down-field by 1.0-4.7 ppm and the signal of the ortho-carbon upfield by 0.8-3.6 ppm.

^{13}C-n.m.r. spectroscopy is not generally the method of choice for distinguishing various classes of flavonoids, it may be of use in some spectral cases. The different types of aglycones are not distinguishable on the basis of the aromatic carbon resonances alone, but chemical shifts for the central three carbons are often quite distinctive (Table-1).

Table-1: Carbon-13 resonances for ring C in flavonoid*.

	C-2	C-3	C=O
Chalcones	136.2-145.4**	116.6-128.1**	188.6-194.6
Flavanones	75.0- 80.3	42.8- 44.6	189.5-195.5
Flavones	160.5-165.0	103.0-111.8	176.3-184.0
Flavonols	145.0-150.0	136.0-139.0	172.0-177.0
Isoflavones	149.8-155.4	122.3-125.9	174.5-181.0
Aurones	146.1-147.7	111.6-111.9 (=CH-)	182.5-182.7

*Data for $CDCl_3$ or $CDCl_3$/DMSO-d_6 solutions except for flavonols.

**For chalcones, C-2 and C-3 represent the C-β and C-α, respectively.

UltraViolet Spectroscopy

The use of u.v. spectroscopy in the structure determination of flavonoid has extensively been studied by L. Jurd[57] and T.J. Mabry[41]. It is extremely helpful in differentiating various class of flavonoids as well as in the location of hydroxyls in the flavonoid nucleus. In general, a flavonoid exhibitstwo bands in the region 300-380 nm (Band I) and 240-270 nm (Band II). The position and intensities of these bandscan be utilized in determining the structure of an unknown flavonoid. Band I is associated chiefly with the absorption in the ring B as well as C_3-portion of the molecule, while band II with the absorption of the benzoyl moiety.

The u.v. spectra of flavones and flavonols in methanol exhibit band I at 300-400 nm and band II at 240-280 nm and can easily be differentiated from other flavonoid groups. Flavanones, flavanonol and isoflavone, though absorb in the range quite different from others, can not be differentiated from each other with the help of u.v. spectra. The methanol spectra of flavanones, flavanonols and isoflavones display a short band in the region at 300-380 nm and a strong band II at 245-295 nm. The u.v. spectra of chalcones and aurones are very similar to each other but quite different from others. Band I in the u.v. spectra of chalcones appears as the strong band in the region 340-390 nm and band II at 220-270 nm. The two bands in the u.v. spectra of aurones appear in the region 370-430 (Band I) and 220-270 nm (Band II).

The relative shift of the two absorption maxima in presence of various shift reagents in methanol is found very useful in locating the hydroxyl functions in the flavonoid molecule. The presence of ortho-dihydroxyl function in the flavonoid can be detected by observing the shifts in the two bands in the presence of sodium acetate-boric acid and also by the comparison of the u.v. spectra in methanol in the presence of $AlCl_3$ and $AlCl_3$-HCl. The confirmation of a hydroxyl function at C-7 of various flavonoids (C-2' of a chalcone) can be made by the relative shift in the band II in the presence of $AlCl_3$-HCl. The presence of a hydroxyl at C-7 portion (C-4' of a chalcone) of various flavonoids can be made with help of sodium acetate. The sodium acetate shift also helps in locating the C-4' hydroxyl (C-4 of chalcone) in various flavonoids. The shift in the band I in the u.v. spectra of various flavonoid in the presence of sodium methoxide is fruitful in determining the hydroxyl at C-4' (C-4 of chalcone). In some cases, however, the sodium methoxide shift can be used in determining the presence of 3',4'-hydroxyls in the nucleus.

The u.v. spectra of the biflavonyls are very similar to those of the monoflavonoids with the only difference that the molecular extinction coefficient (ϵ) of biflavones approximately double as compared to the corresponding monoflavonoid unit. This demonstrates the presence of two isolated chromophores of flavonoids per molecule of a biflavonoid.

Discussion

The Garcinia genus (family Guttiferae) consists of 180 species, mostly evergreen trees and occasionally shrubs distributed in tropical Asia, Africa and Polynesia. Ten major and twenty closely allied species are reported to occur in India[58].

Garcinia species have long been used in East and South East Asia and Africa in the treatment of dysentery, diorrhea and wounds[59]. A preparation of G. xanthochymus Hook. f. is reported to be used in the treatment of bilions conditions, diorrhea and dysentery. The gum resin of G. hanburyi Hook. f. is a drastic purgative, an emetic and vermifuge to treat tapeworm which, in too large doses, can be fatal.

Among the natural products isolated from this genus, the 3/8" linked biflavonones, isolated from G. kola, have been reported to possess significant antiheptotoxic properties[60] as evidenced by liver protection of laboratory animals challenged with phalloidin, a known liver toxin.

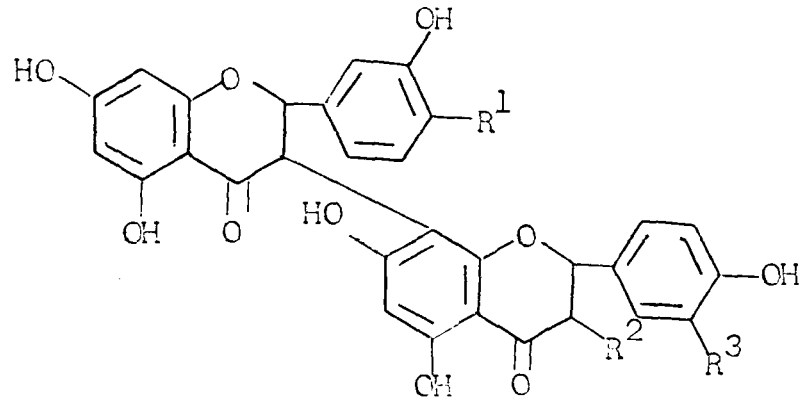
The genus has been the subject of a considerable amount of phytochemical investigation which has revealed it to be a major source of prenylated xanthones[61], benzophenones[62] and of biflavonoids[63] linked between C-3 and C-8.

The biflavonoids, isolated from almost all the species of genus Garcinia worked out so far, comprise of either two flavanone or a flavanone-flavone units. There is no report on the

isolation of a 3/8" linked flavone-flavone biflavonoid from plants. The 'GB' biflavanones, so called because they are major constituents of the heartwood of G. buchananii Baker, were first isolated in 1967 by Jackson et al.[64] Only five members of 3/8" flavanone-flavone family have been isolated from this genus so far. Morelloflavone, the first to be discovered, was isolated from the heartwood of G. morella by Venkataraman et al. in 1967[65].

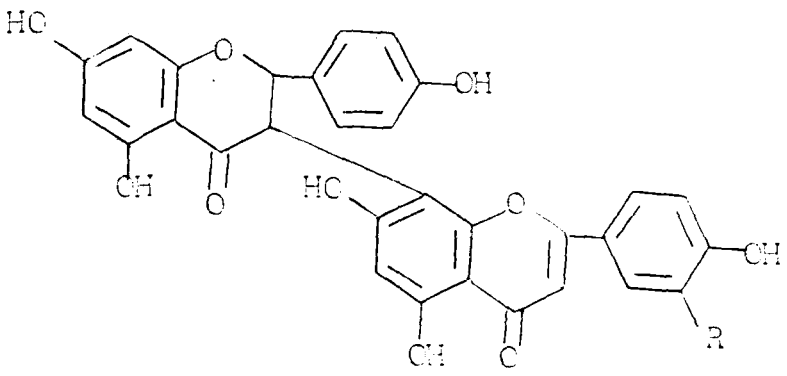
The work on the isolation of flavonoids from plants has been brilliantly reviewed by Harbone and Mabry first in 1975[6] and recently in 1982[66]. It is not, therefore, our aim to review the work done on the genus Garcinia but an attempt is made to describe the hydroxylation pattern found in naturally occurring 3/8" -linked biflavonoids. The ring A in all the 3/8"-biflavonoids, isolated so far, possesses three alternate oxygens or is drived from Phloroglucinol. The hydroxylation pattern in ring B is also typical of monoflavonoids. The ring B generally has a para-hydroxyl formation or 3,4-dihydroxyl grouping. The hydroxyl group is often present at C-3. Figure-13 shows the various hydroxylation patterns found in 3/8"-biflavonoids.

G. nervosa Mig. (Syn. G. andersoni Hook. f.) is found in Eastern Penisula, Malacca and Nigeria. The leaves of the plant are very large 1-2 ft oblong, thickly coriaceous, subacute rounded or cordate at the base leaving numerous strong nerves[83].



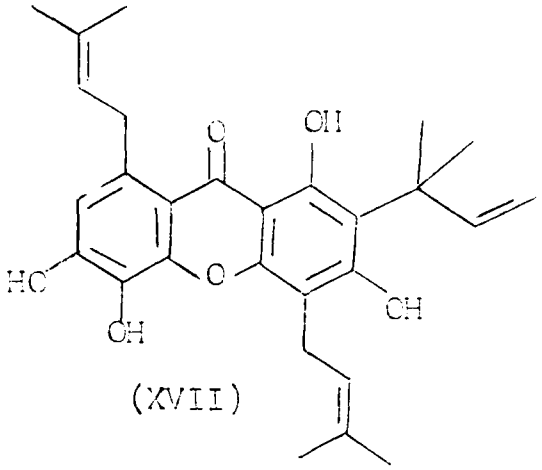
		R^1	R^2	R^3
1.	GB-1a[60,63,67-75]	H	H	H
2.	GB-1[60,63,67-70,72,74-77]	H	OH	H
3.	GB-2a[60,67,68,72,73,75]	H	H	OH
4.	GB-2[60,63,68,6972,74-76]	H	OH	OH
5.	Manniflavanone[72]	OH	OH	OH

Fig.-13



		R
1.	Volkensiflavone (BGH-III=talbotiflavone)[69,70,73,76,78,79]	H
2.	Fukugetin (BGH-II=morelloflavone)[67,69,70,73,75,76,78-82]	OH
3.	3-O-methyl fukugetin[76,80-82]	OMe

The work on the stem bark of this plant has recently been performed and a new xanthone named nervosaxanthone (XVII) has been isolated[84]. No biflavonoid was obtained from this part of the plant.



(XVII)

The leaves of G. nervosa were collected from Zaria, Nigeria and the present discussion deals with the isolation of biflavonoids from the ether soluble part of ethanolic extract of leaves of G. nervosa. Four biflavonoids have been isolated and purified by modern techniques, such as column chromatography and preparative thin layer chromatography. This discussion deals with the characterisation of one of the isolated biflavonoids by chemical and spectroscopic methods, such as i.r., u.v., ^{1}H-n.m.r., ^{13}C-n.m.r. spectroscopy and mass spectrometry. The structural elucidation of remaining biflavonoids is in hand.

The column chromatography of the ether soluble part over silica gel was performed and the column was eluted successively with petrol, petrol:benzene mixtures, benzene, benzene:ethyl acetate mixtures and finally with ethyl acetate. The column

chromatography did not prove fruitful as no compound could be obtained in pure form. Instead, the complex mixture was resolved into mixture of two and three compounds. Preparative-TLC was, then, performed using benzene:pyridine:formic acid (36:9:5, 40:10:2, 100:20:7), toluene:ethyl formate:formic acid (5:4:1), chloroform:MeOH (19:1, 17:3), benzene:ethyl acetate (1:1) as the developer. The results with the BPF were not encouraging and although the result of the TEF system were better, this was avoided as the compounds were acid and base sensitive. The compounds were finally obtained in pure form by the preparative-TLC over silica gel using benzene:ethyl acetate (1:1) system. Four compounds coded as GN-1, GN-2, GN-3 and GN-4 isolated were crystallized from methanol and benzene.

GN-1 was crystallized from methanol-benzene as yellow crystals, m.p.232-234^{o}C. It gave pink colour with Zn-HCl and Mg-HCl, suggesting it to be a flavonoid. It gave positive $FeCl_3$ test indicating the presence of phenolic hydroxylic group/s. The infra-red spectrum of the compound showed absorptions at 3300 cm^{-1} (OH), 1640 cm^{-1} (5-hydroxyflavanone) and 1610 cm^{-1} (5-hydroxy-flavone). The compound was methylated with dimethyl sulphate-potassium carbonate in acetone to give GNMe-1, m.p.123-125^{o}C. The i.r. spectrum of GNMe-1 possessed bands at 1645 and 1675 cm^{-1}. Such spectral changes on methylation are reminiscent of the behaviour of flavanone and flavone systems, respectively, bearing

hydroxy group at C-5. The compound GN-1 was acetylated with acetic anhydride-pyridine to give GNAc-1, m.p.145-149°C. The i.r. spectrum of GNAc-1 displayed bands at 1770 (CH_3-$\overset{O}{C}$-) and 1645 cm^{-1} (broad).

The ultra-violet spectrum of GN-1 showed absorption maxima in the region 255 sh, 265 and 320 nm. The shifts of the absorption maxima in the presence of NaOMe, $AlCl_3$, NaOAc, NaOAc-H_3BO_3 and $AlCl_3$-HCl were also studied but did not prove helpful as the bands of the two units were merged. The structure was elucidated with the help of ^{1}H-n.m.r. and ^{13}C-n.m.r. spectroscopy and mass spectrometry.

The decoupled ^{13}C-n.m.r. spectral data of GN-1 displayed thirty signals confirming it to be a biflavonoid. ^{13}C-n.m.r. spectrum displayed in the lowest field, two signals at ∂ 196 ..... and 181 ..... ppm which confirmed the presence of a flavanone and a flavone unit in the molecule. The possibility of a chalcone unit was ruled out in view of the other spectral data. Ten signals in the region 145.5789-166.4430 ppm were assignable to oxygenated aromatic carbons. The signals for the non-oxygenated aromatic carbons were seen in the range 95.2061-128.6528 as fourteen singlets. Two signals at ∂ 80.8783 and 48.2935 were attributed to C-2 and C-3, respectively of the flavanone unit. The assignment of the ^{13}C-n.m.r. data was made by comparison with the ^{13}C-n.m.r. data of monoflavonoids[56] in table-2.

Table-2: ^{13}C-n.m.r. chemical shifts (ppm) for flavanone and flavone.

	Flavanone	Flavone
C-4	196.0443	181.5555
C-7	166.4430	163.4713
C-5	163.7341	160.4599
C-9	162.7835	155.2040
C-4'	149.6122	157.2827
C-3'	145.5789	114.3888
C-1'	128.3528	121.0819
C-6'	119.1760	128.3163
C-5'	116.0779	114.3888
C-2'	113.2590	128.1014
C-10	103.1420	102.2117
C-6	96.1393	98.5595
C-8	95.2061	100.4886
C-2	80.8783	161.5706
C-3	48.2935	101.4628

^{13}C-n.m.r. of GM-1

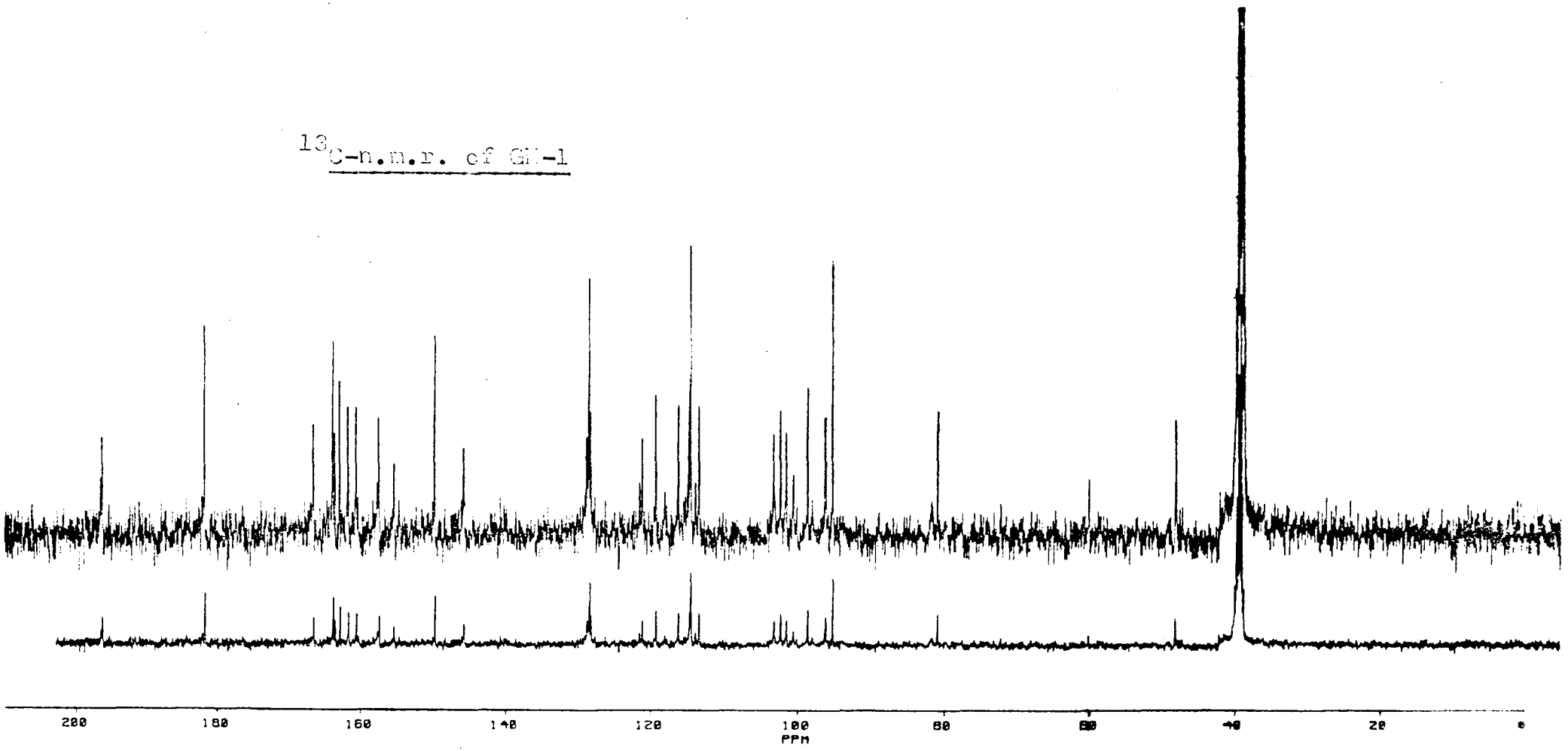


Fig.-14

The ^{1}H-n.m.r. spectrum of GN-1 in DMSO-d_6 confirmed the absence of methoxyl group. It can be said, therefore, in view of the ^{13}C-n.m.r. data that only hydroxyl groups are present in the molecule. The presence of seven hydroxyl group was confirmed by the seven singlets, each for one proton, in the lowest field of the spectrum at δ 9.27, 9.50, 9.83, 10.79, 11.18, 12.20 and 13.20. A pair of doublets (J=12 Hz) at δ 5.64 and 4.83 were assignable to C-2 and C-3 protons, respectively of the flavanone unit. The downfield shift of the C-3 proton relative to the chemical shift in flavanone suggested the involvement of C-3 of flavanone in the interflavonoid linkage. An upfield singlet in the aromatic region at δ 5.91 for two protons was attributed to two protons on the ring A of flavanone unit. Another singlet at δ 6.17 for one proton was assignable to the proton on the ring A of flavone unit suggesting the linkage of the other unit through ring A. A pair of doublets (J=8 Hz) in the aromatic region at δ 6.32 (H-3''',5''') and 7.08 (H-2''',6'''), each for two protons, was indicative of the presence of a hydroxyl group at C-4' of either of the B rings. A singlet at δ 6.53 for one proton was assigned to C-3 proton of the flavone unit. A doublet (J=8 Hz) for one proton at 6.85 (H-5') and a multiplet for two protons at δ 7.37 (H-2',6') confirmed the presence of a 3',4'-dihydroxyl system in the other B ring.

It can be said, in view of the above discussion, that compound GN-1 is a biflavonoid having a flavanone and a flavone unit. Also, the rings A in both the units are derived from

^{1}H-n.m.r. of GN-1

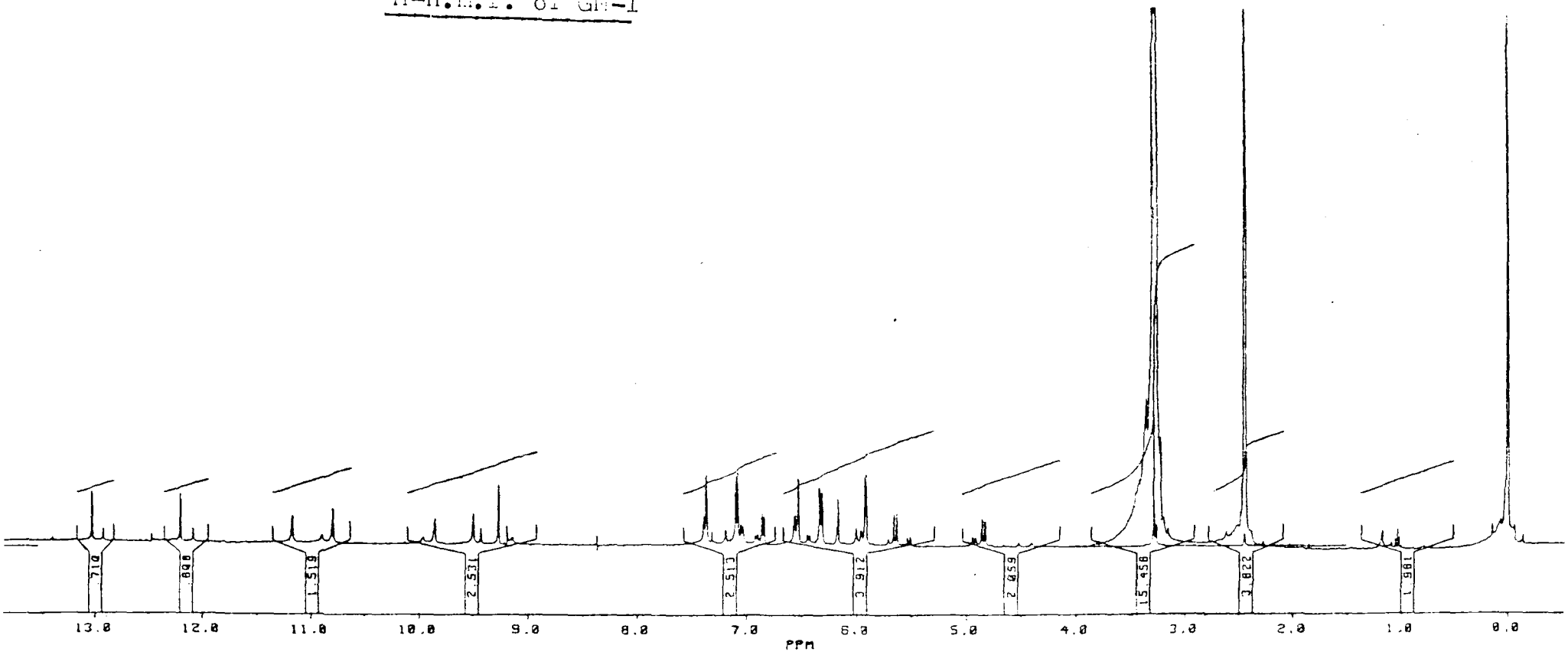


Fig.-15(a)

^{1}H-n.m.r. of GK-1 (Expansion)

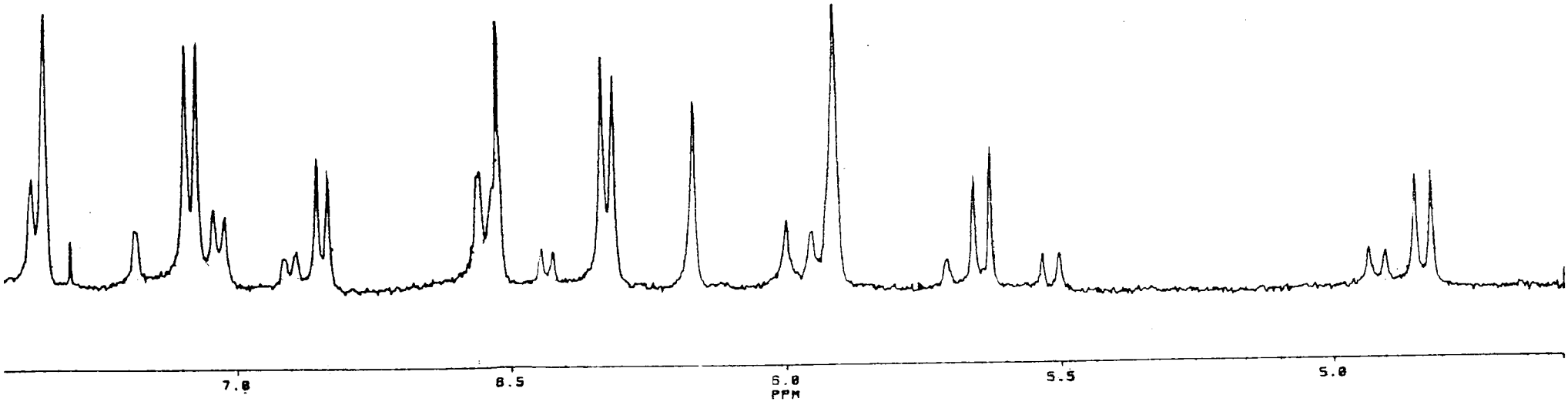


Fig.-15(b)

^{1}H-n.m.r. of GN-1 (Expansion)

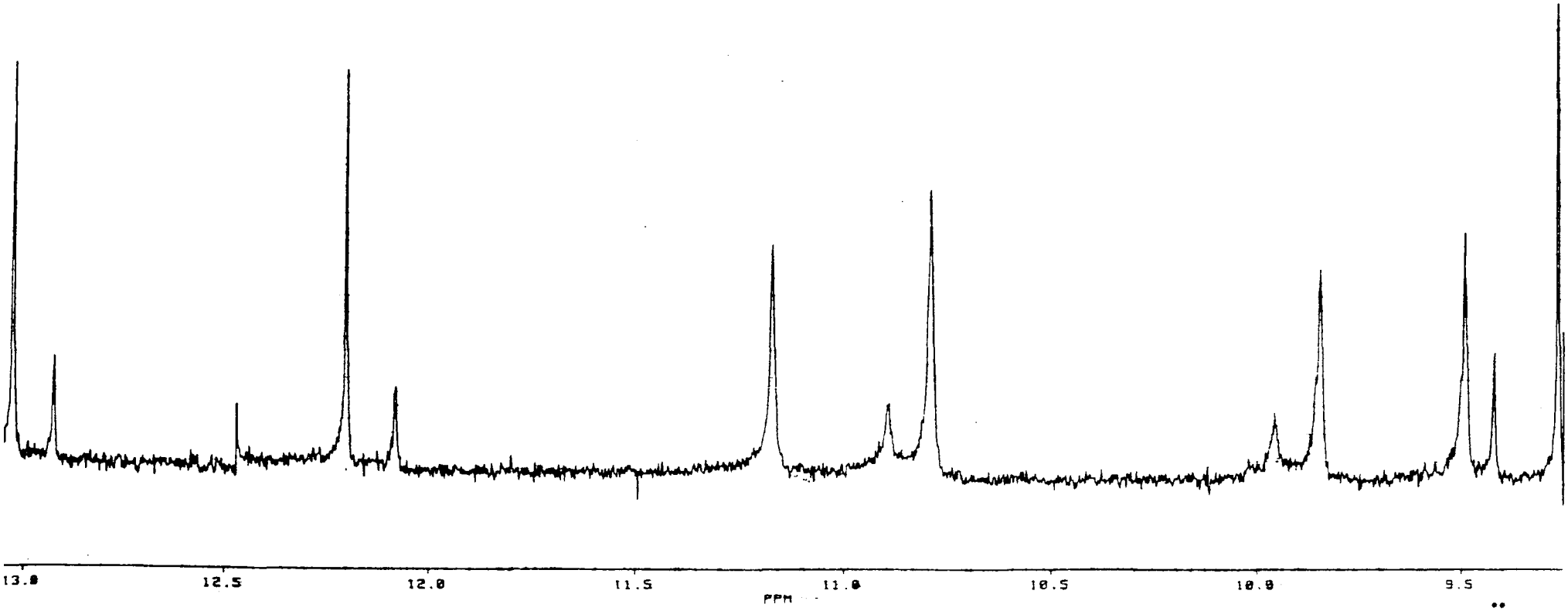
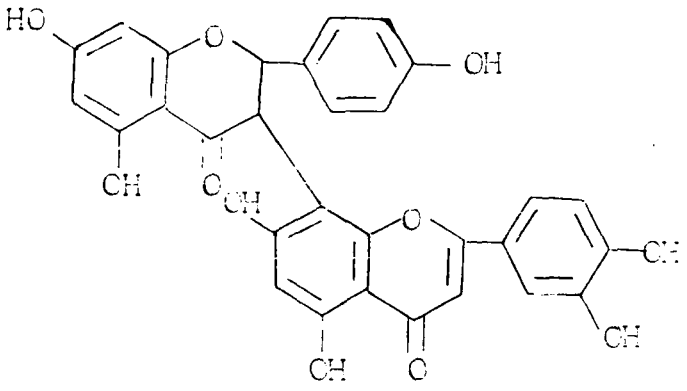
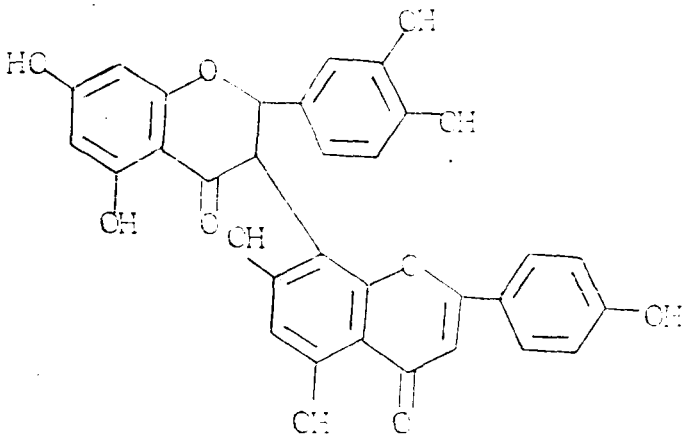


Fig.-15(c)

phloroglucinol and the C-3 of flavanone is linked to C-8 of flavone unit. The only thing which is to be determined is the nature of B rings of the two units. There are now two possible assignments for the compound GN-1 i.e. either (XVIII) or (XIX). The compound (XVIII) is reported in the literature and its melting point 300°C is much higher than that of GN-1. The probable structure of the compound is thus (XIX), which was finally confirmed with the help of mass spectral data.



(XVIII)



(XIX)

The mass spectrometry which is one of the best techniques for determining the structure of biflavonoids and especially the interflavonoid linkage proved helpful in our case and provided evidences in favour of the interflavonoid linkage as well as the nature of the two B rings. As is reported for C-3/C-8 linked biflavonoids, the mass spectrum of GN-1 did not exhibit many of the characteristic peaks and the most significant peak was observed

at m/z 126[72]. The molecular ion peak could not be observed for GN-1. The mass spectra of methyl ether and acetyl derivatives also did not show molecular ion peaks and though seven spectra of methyl ether (GNMe-1) were recorded, in none could the peaks above m/z 200 be detected.

As discussed in the theoretical part of this dissertation, the major fragmentation path of the flavonoids is RDA and RDA by pathway I, which gives both A and B fragments, is most significant. It is not clear why many important RDA fragments could not be observed in the case of GN-1 and its derivatives, but the major fragmentation path was the loss of ring A of flavanone unit giving a base peak at m/z 126.

The problem of confirming the nature of two B rings was, however, solved with the help of mass spectral data of GN-1. The most significant peak in the case of GN-1 was at m/z 110, which could only be assigned to the dihydroxyl phenyl cation. Another diagnostic fragment at m/z 418 may be explained in terms of loss of ring B of flavanone. The formation of an ion at m/z 260 can be explained from the $M^{+\bullet}$ by the RDA of both the units accompanied by the loss of a CO molecule. The peaks at m/z 152 and 153 are due to the ring A fragments of RDA of flavanone unit. RDA by pathway II of the flavone unit and loss of phloroglucinol ring from the flavanone unit can explain the peak at m/z 270. The peaks at m/z 286 and 287 can be explained by the RDA of both the

units. The peak at m/z 242 may be due to ion formed by the loss of CO from ion at m/z 270. A peak at m/z 328 may be explained in terms of RDA of flavone unit and loss of ring B from flavanone unit. The peaks at m/z 149 and 150 may be due to the doubly charged ions formed by the loss of ring B from flavanone unit and RDA followed by loss of CO from flavone unit.

The above discussion provides sufficient evidence in favour of the 3/8''-linkage and dihydroxylated B ring of flavanone unit and monohydroxylated ring B of flavone unit. The compound GN-1 was, therefore, characterised as 5,7,5'',7'',3',4',4'''-hepta-hydroxy-[I-3,II-8'']-flavononyl flavone (XIX). The structure elucidation of other compounds, GN-2, GN-3 and GN-4, is in hand.

EI-MS of GN-1

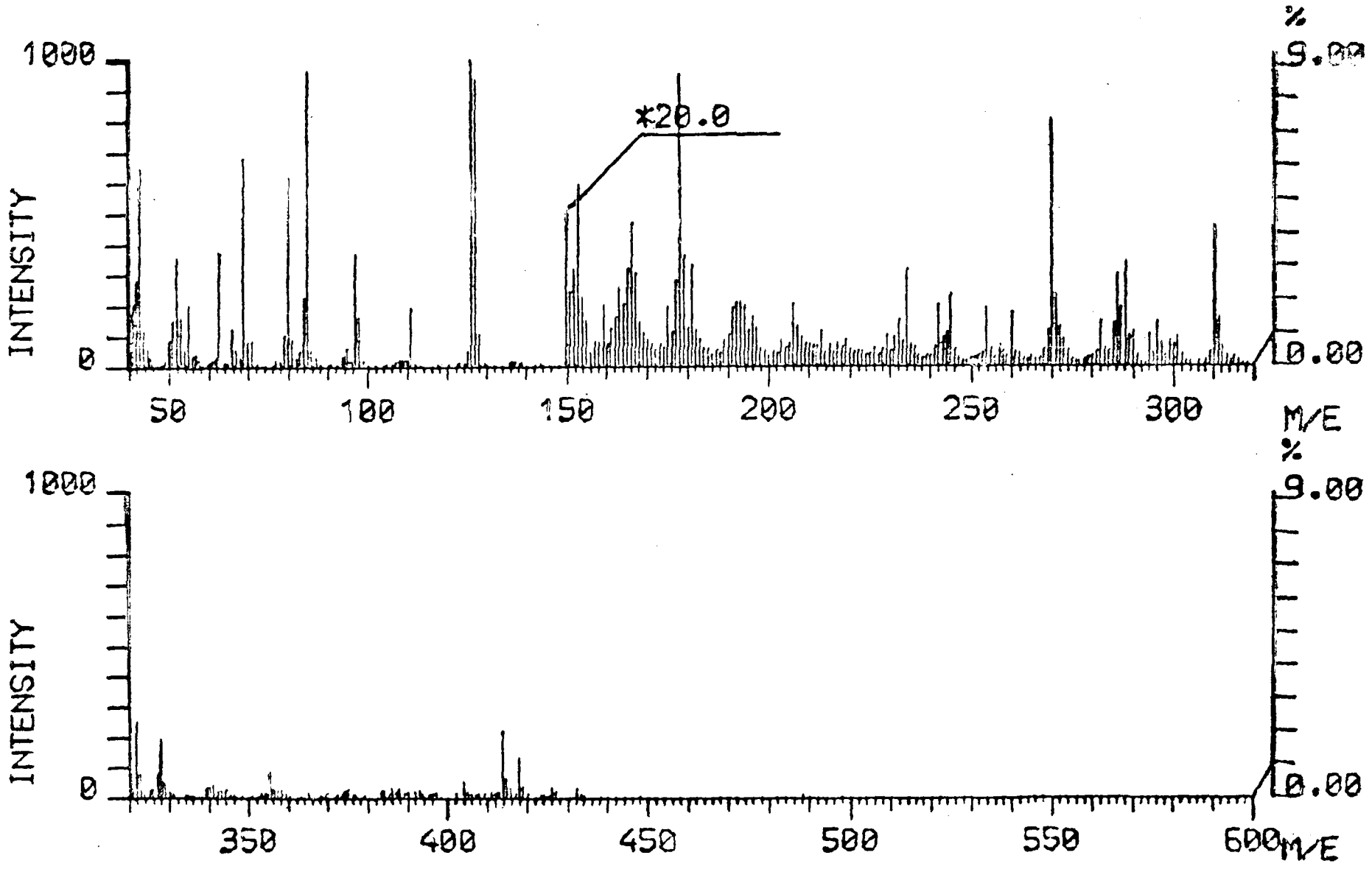


Fig.-16

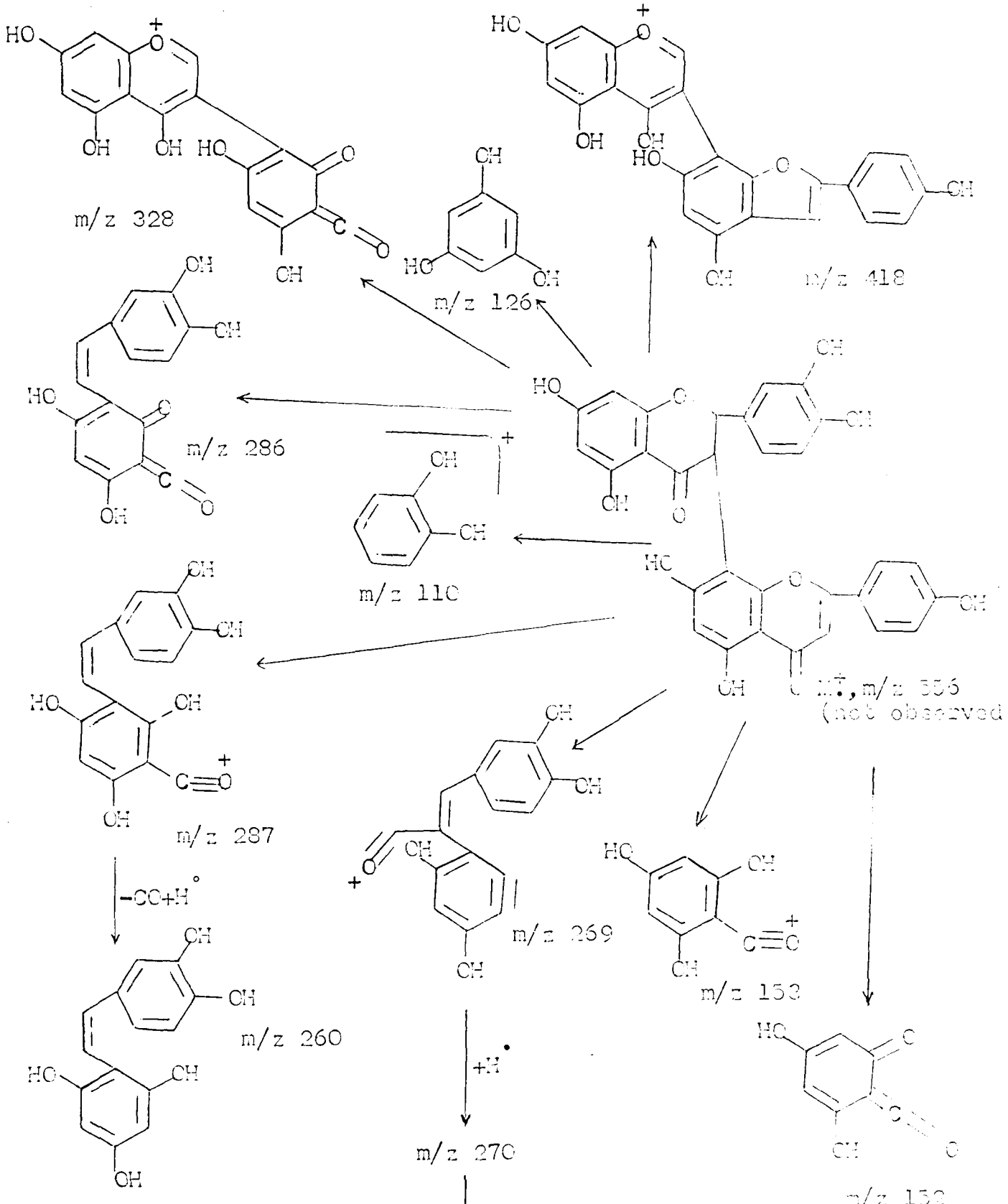

m/z 328
m/z 126
m/z 418
m/z 286
m/z 110
M+, m/z 556
(not observed
m/z 287
m/z 269
m/z 153
-CO+H°
m/z 260
+H°
m/z 270
-CO
m/z 242
m/z 152

Chart-III

Experimental

Extraction of the leaves of Garcinia nervosa Mig. (Guttiferae)

Leaves (3 Kg) of G. nervosa, procured from Zaria, Nigeria, were extracted several times with petrol (60-80°) by cold percolation. The treated leaves were crushed and defatted again by repeated extraction with petrol and benzene successively, followed by boiling alcohol. The combined alcohol extracts were concentrated first at atmospheric pressure and then under reduced pressure. A dark viscous residue was obtained. This was treated successively with boiling petroleum ether (60-80°) and benzene to remove fatty and resinous matter. The dark brown residue (700 gm), thus obtained and responded to the usual colour test for flavonoids.

Purification of flavonoid mixture-column chromatography

The column chromatography of the ether soluble part over silica gel was performed and the column was eluted successively with petrol, petrol-benzene mixture, benzene, benzene-ethyl acetate mixtures and ethyl acetate. The column chromatography did not prove fruitful as no compound was isolated in pure form. Instead, the complex mixture was resolved into mixture of two and three compounds.

Separation of flavonoidic mixture-preparative thin layer chromatography

Using a thin layer spreader, glass plates (20x20 cm) were coated with a well stirred suspension of silica gel (BDH) to give a layer approximately 0.5 mm in thickness. After drying at room temperature, the plates were activated at 120°C for at least two hours.

The brown mass obtained was dissolved in acetone and the solution was applied to plates with the help of a mechanical applicator, 2 cm from the lower edge of the plates. The plates mounted on a steel frame were placed in a Desaga glass chamber (45x22x25 cm) containing 500 ml of developing solvent (benzene: ethyl acetate, 1:1). When the solvent front travelled 15 cm from the starting line, the development was interrupted and the plates were dried at room temperature. The position of the bands were marked in U.V. light. The marked pigment zones were scraped with the help of a nickel spatula and eluted separately in soxhlets with acetone. The solvent was recovered. The four bands, thus obtained, were labelled as GN-1, GN-2, GN-3 and GN-4.

GN-1

It was crystallized as pale yellow crystals (950 mg) from MeOH-benzene, melting point 232-234°C.

I.R. Spectra of GN-1

KBr : ν_{max} 3300, 1745, 1640, 1610, 1510, 1460, 1380 1260, 1160, 1030 and 835 cm^{-1}.

U.V. Spectra (λ_{max}, nm) of GN-1

MeOH	:	255 sh, 265, 320
NaOMe	:	258, 298, 362 sh, 385
$AlCl_3$	:	255, 285, 405
$AlCl_3$/HCl	:	255, 282, 335, 365
NaOAc	:	260, 300, 355 sh
NaOAc/H_3BO_3	:	245, 265, 305, 350

^{1}H-N.M.R. (DMSO-d_6, 400 MHz) of GN-1

δ 4.83 (1H, d, J=16 Hz, H-3); 5.64 (1H, d, J=16 Hz, H-2); 5.91 (2H, s, H-6,8); 6.17 (1H, s, H-6); 6.32 (2H, d, J=8 Hz, H-3'',5''); 6.53 (1H, s, H-3); 6.84 (1H, d, J=8 Hz, H-5'); 7.08 (2H, d, J=8 Hz, H-2'',6''); 7.37 (2H, m, H-2',6'); 9.27, 9.50, 9.83, 10.79, 11.18, 12.20 and 13.20 (1H each, s, HO-5,7,5 ,7 ,3',4',4'').

^{13}C-N.M.R. (100 MHz, DMSO-d_6) of GN-1

The assignment of the ^{13}C-n.m.r. data was made by comparison with the ^{13}C-n.m.r. data of monoflavonoids and the ^{13}C-n.m.r. table is mentioned in table-2 of discussion.

Mass (MI-MS) of GN-1

MS(rel.int.) : m/z 418 (0.75); 328 (1.00); 287 (1.80); 286 (1.50); 270 (4.00); 260 (0.90); 242 (1.00); 153 (2.90); 152 (1.50), 126 (100.00); 110 (20.00).

Acetylation of GN-1

The GN-1 (100 mg) was dissolved in pyridine (4.0 ml) and acetic anhydride (8.0 ml) was added. The resulting mixture was heated on a water bath for 8 hrs. It was cooled-down to room temperature and poured over crushed ice. The separated solid was filtered, washed several times with cold water and dried. On crystallization from EtOH-water, solid crystals (60 mg) were obtained, m.p.145-149^{o}C.

I.R. Spectra of GNAc-1

KBr : ν_{max} 1770, 1645 (broad), 1500, 1370, 1190, 1120, 1020 and 900 cm^{-1}.

Methylation of GN-1

GN-1 (100 mg), anhydrous potassium carbonate (3 gm), dimethyl sulphate (0.5 ml) and dry acetone (400 ml) were refluexed on water-bath for 24 hrs. Refluxing continued till it gave negative alc. $FeCl_3$ test. It was then filtered and residue washed several times with hot acetone. The filtrate and washings were combined and evaporated to dryness. The yellow residue was treated with petroleum ether to remove any unused dimethylsulphate and then dissolved in chloroform (25 ml). The chloroform solution was washed several times with water, dried (Na_2SO_4) and evaporated to give crude solid. The solid was purified by column chromatography. On crystallization with $CHCl_3$-MeOH, it gave white crystalline solid, m.p.123-125°C.

I.R. Spectra of GNMe-1

KBr : ν_{max} 1675, 1645, 1600, 1515, 1460, 1375, 1260, 1155, 1110, 1040, 1020 and 830 cm^{-1}.

^{1}H-N.M.R. ($CDCl_3$, 60 MHz) of GNMe-1

δ 3.68, 3.74, 3.80, 3.84, 3.88, 3.90 and 3.94 (3H, each s, CH_3-O-5,7,5 ,7 ,3',4',4''); 4.91 (1H, d, J=13 Hz, H-3); 5.86 (1H, d, J=13 Hz, H-2); 6.20 (2H, s, H-6,8); 6.30 (1H, s, H-6); 6.50 (1H, s, H-3); 6.60 (2H, d, J=8 Hz, H-3'',5''); 6.98 (1H, d, J=9 Hz, H-5'); 7.1-7.4 (4H, m, H-2'',6'',2',6').

Bibliography

1. T.A. Geissman, 'Chemistry of Flavonoid Compounds', Pergamon Press, (1962), (a) p.468; (b) p.546.

2. K. Venkataraman, in 'Progress in the Chemistry of Organic Natural Product', L. Zeechmeister (Ed.), Springer-Verlag, New York, (1959), (a) p.2; (b) p.46.

3a. C.H., Lea and P.T.A. Swoboda, Chem. and Ind., 1429 (1956).

b. A.C. Mehta and T.R. Seshadri, J. Sci. Ind. Res., 18B, 24 (1959).

4. S.M. Kupchan, C.W. Sigel, R.J. Hemingway, I.R. Know and M.S. Udayamurthy, Tetrahedron, 25, 1603 (1969).

5. C.P.S. Jagat, S. Singh, H.S. Garg, N.M. Khanna and M.M. Dhar, Phytochemistry, 15, 232 (1976).

6. J.B. Harborne, T.J. Mabry and H. Mabry, 'The Flavonoids', Chapman and Hall, London, (1975), (a) p.693; (b) p.82.

7. J.B. Harborn, 'Comparative Biochemistry of Flavonoids', Academic Press, London, (1967), p.251.

8. H. Grisebach and W. Barz, Naturwiss., 56, 533 (1969).

9. H. Pacheco, Bull. Soc. Franc. Physiol. Vegetable, 15, 3 (1969).

10. A.J. Birch and F.M. Dorrovan, Aust. J. Chem., 6, 360 (1953).

11. H. Grisebach, Z. Naturf., 12b, 227,697 (1957); ibid., 13b, 335 (1958); ibid., 14b, 485 (1959).

12. T.A. Geissman and T. Swain, Chem. and Ind., 984 (1957).

13. H. Grisebach, Planta Med., 10, 385 (1962).

14. H. Grisebach and G. Brandner, Biochem. Biophys. Acta, 60, 51 (1962).

15. F. Kreuzaler and H. Hahlbrock, FEBS Letters, 28, 69 (1972).

16. K. Hahlbrock and H. Grisebach, in 'The Flavonoid', J.B. Harborne, T.J. Mabry and H. Mabry (Ed.), Chapman and Hall, London, (1975), p.866.

17. H. Grisebach and L. Patschke, Z. Naturf., 16b, 645 (1961).

18. H. Grisebach and G. Brandner, Z. Naturf., 16b, 2 (1961).

19. H. Grisebach and W. Bilhuber, Z. Naturf., 22b, 746 (1967).

20. L. Patschke, W. Barz and H. Grisebach, Z. Naturf., 19b, 110 (1964).

21. F.M. Dean, 'Naturally Occurring Oxygen Ring Compounds', Butterwoth, London, (1963), p.335.

22. E. Wong and E. Moustafa, Tetr. Lett., 3021 (1966).

23. H. Grisebach and W.D. Ollis, Experientia, 17, 4 (1961).

24. G. Litkei and R. Bognar, Acta. Chim. Hung., 77, 93 (1973).

25. J. Alger and J.P. Flynn, Proc. Roy. Irish. Acad., 42b, 1 (1934).

26. T. Oyamada, J. Chem. Soc.(Japan), 55, 1256 (1934).

27. A.J. Birch, in 'Chemical Taxonomy', T. Swain (Ed.), Butterworth, London, (1963), Chap. 6.

28. J.D. Bu'Lock, 'Biosynthesis of Natural Products', Mc Graw-Hill, New York, (1965), (a) p.90; (b) p.86.

29. A. Pelter, J. Bradshaw and R.F. Warren, Phytochemistry, 10, 835 (1971).

30. D.G. Roux and D. Ferreira, Phytochemistry, 13, 2039 (1974).

31. E.W. Underhill, J.E. Watkin and A.C. Neish, Can. J. Biochem. Physiol., 35, 219 (1957).

32. E. Wong, Chem. and Ind., 1985 (1954).

33. J.P. Van Der Merwe, D. Ferreira, E.V. Brandt and D.G. Roux, J. Chem. Soc. Chem. Commun., 521 (1972).

34. F. DuR. Volsteedt, G.J.H. Rall and D.G. Roux, Tetr. Lett., 1001 (1973).

35. P. Monitto, 'Biosynthesis of Natural Product', Ellis Horwood Ltd. Publisher, (1981), p.410.

36. J.W. Clark-Lewis and D.C. Skingle, Aust. J. Chem., 20, 2196 (1967).

37. J.W. Clark-Lewis and R.W. Jamison, Aust. J. Chem., 21, 2247 (1968).

38. L. Jurd, Chem. and Ind., 2175 (1967); Tetrahedron, 23, 1057 (1967).

39. E. Haslam, Phytochemistry, 16, 1625 (1977).

40. H.D. Locksley, in 'Progress in the Chemistry of Natural Product', L. Zeichmeister (Ed.), Springer-Verlag, New York, (1973), p.207.

41. T.J. Mabry, K.R. Markham and M.B. Thomas, 'The Systematic Identification of Flavonoids', Springer-Verlag, New York, (1970).

42. T.J. Batterham, 'NMR Spectra of Simple Heterocycles', Wiley, New York, (1973), p.385.

43. T.J. Mabry, J. Kagan and H. Roster, Monograph, 'Nuclear Magnetic Resonance Analysis of Flavonoid', Univ. of Texas Publication, No.6418 (1964).

44. J. Massicot and J.P. Marthe, Bull. Soc. Chim., France, 1962 (1962).

45. J.W. Clark-Lewis, L.M. Jackson and T.M. Spotswood, Aust. J. Chem., (a) 17, 632 (1964); (b) ibid., 31, 2059 (1968).

46. M. Okigawa, N. Kawano, W. Rahman and M.M. Dhar, Tetr. Lett., 4125 (1972); M. Okigawa, N.U. Khan, N. Kawano and W. Rahman, Chem. and Ind., 575 (1974); J. Chem. Soc., 1563 (1973).

47. A. Pelter, R. Warren, J.W. Usmani, R.H. Rizvi, M. Ilyas and W. Rahman, Experientia, 25, 350 (1969).

48. T.J. Batterham and R.J. Highet, Aust. J. Chem., 17, 428 (1964).

49. A.C. Waiss, Jr., R.E. Lundin and D.J. Stern, Tetr. Lett., 513 (1964).

50. R.G. Wilson and D.H. William, J. Chem. Soc.(C), 2477 (1968).

51. D.G.I. Kingston, Tetrahedron, 27, 2691 (1971).

52. R.I. Reed and J.M. Wilson, J. Chem. Soc.(C), 5949 (1963).

53. A. Pelter, P. Stainton and M. Barber, J. Heterocyclic Chem., 2, 262 (1965).

54. H. Andier, Bull. Soc. Chim., France, 2892 (1966).

55. C. Van de Sande, J.W. Serum and M. Vanderwalle, Org. Mass Spectrom., 6, 1333 (1972).

56. KenR. Markham and V. Mohan Chari, in 'The Flavonoids: Advances in Research', J.B. Harborne and T.J. Mabry (Ed .), Chapman and Hall, London, (1982), p.19.

57. L. Jurd and R.M. Horowitz, J. Org. Chem., 22, 1618 (1957).

58. The Wealth of India, CSIR, New Delhi, India, (1956), Vol.IV, p.99.

59. L.M. Perry and J. Metzger, 'Medicinal Plants of East and South-East Asia', MIT Press, London, (1980), p.175.

60. M.M. Iwu, Experientia, 41, 699 (1985).

61. P. Yates and G.H. Stout, J. Am. Chem. Soc., 80, 1691 (1958).

62. C.G. Karanjgaokar, A.V. Rama Rao, K. Venkataraman, S.S. Yemul and K.J. Palnur, Tetr. Lett., 4977 (1973).

63. B. Jackson, H.D. Locksley, F. Scheinmann and W.A. Wolstenholme, J. Chem. Soc., 3791 (1971).

64. B. Jackson, H.D. Locksley, F. Scheinmann and W.A. Wolstenholme, Tetr. Lett., 787 (1967).

65. C.G. Karanjgaokar, P.V. Radhakrishnan and K. Venkataraman, Tetr. Lett., 3195 (1967).

66. J.B. Harborne and T.J. Mabry, 'The Flavonoid: Advance in Research', Chapman and Hall, London, (1982).

67. A.A.L. Gunatilaka, A.M.Y. Jasmin De Silva, S. Sotheeswaran, S. Balasubramanian and M.I.M. Wazur, Phyto., 23, 322 (1984).

68. A.A.L. Gunatilaka, H.T. Badra Sriyani, S. Sotheeswaran and E.S. Waight, Phyto., 22, 233 (1983).

69. W.M. Bandaranayake, S.S. Selliah, M.U.S. Sultanbawa and W.D. Ollis, Phyto., 14, 1878 (1975).

70. Baslas et al., Current Sci., 48, 814 (1979).

71. A.V. Rama Rao, M.R. Sarma, K. Venkataraman and S.S. Yemul, Phyto., 13, 1241 (1974).

72. E.G. Crichton and P.G. Waterman, Phyto., 18, 1553 (1979).

73. Fa-Ching Chen, Yuh-Meei Lin and Jeng-Chin Hung, Phyto., 14, 300 (1975).

74. A. Pelter, Tetr. Lett., 19, 1767 (1967).

75. W.H. Ansari, W. Rahman, D. Barraclough, Mrs. R. Maynard and F. Sheinmann, J. Chem. Soc. Perkin-Trans.I, 13, 1458 (1976).

76. M. Konoshima, Y. Ikeshiro and S. Miyahara, Tetr. Lett., 4203 (1970).

77. Iwu et al., J. Natl. Prod., 45, 650 (1982).

78. P.J. Cotterill, F. Scheinmann and G.S. Puranik, Phyto., 16, 148 (1977).

79. Bastas et al., Acta. Cima, Indica[Ser.]Chem., 7, 31 (1981).

80. Y. Ikeshiro and M. Konoshima, Tetr. Lett., 43, 4383 (1972).

81. P.G. Waterman and E.G. Crichton, Phyto., 19, 2723 (1980).

82. G.D. Monache, F.D. Morache, P.G. Waterman, E.G. Crichton and R. Alves De Lima, Phyto., 23, 1757 (1984).

83. J.D. Hooker, 'The Flora of British India', L. Reeve and Co. Press, London, (1872), p.270.

84. S.A. Ampofo and P.G. Waterman, Phyto., 10, 2351 (1986).